ABSTRACT

Title of thesis: TOWARD THE DEVELOPMENT OF INTEGRATED OYSTER-ALGAE AQUACULTURE IN THE CHESAPEAKE BAY

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Oyster aquaculture is a rapidly expanding industry in the Chesapeake Bay. Experiments were conducted to investigate the biogeochemical impact of a commercial oyster aquaculture facility on downstream waters at a facility on Maryland's Eastern Shore. An algal production system (ATS) was installed at the facility to assess the potential for bioremediation and algal production in an integrated multi-trophic aquaculture system (IMTA). Results of the experiments showed an increase in available ammonia downstream of the aquaculture facility, coupled with decreases in dissolved oxygen and total phytoplankton. The algal production system demonstrated an average productivity rate of 82.8 g/m^2*day^{-1}, a nitrogen (N) removal rate of 9.6 gN/m^2*day^{-1}, a phosphorus (P) removal rate of 0.20 gP/m^2*day^{-1}, and harvests consisted of an average of 7.8% organic content. Productivity and N and P removal rates from this study are higher than other systems tested in the Chesapeake Bay region at sites without an aquaculture facility.

TOWARD THE DEVELOPMENT OF INTEGRATED OYSTER – ALGAE AQUACULTURE IN THE CHESAPEAKE BAY

by

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CHAPTER I: Background and Overview

1.1 Overview

The Eastern Oyster (*Crassostrea virginica*) has long been a keystone species in the Chesapeake Bay both commercially and ecologically. Historical overharvesting of oysters in the Chesapeake Bay has reduced abundance, damaged the ecosystem, and led to large-scale efforts to restore their population through regulations on harvesting, the construction of state owned oyster hatcheries, and the emergence of the oyster aquaculture industry.

While restoration programs have focused on increasing the total number of oysters in the Chesapeake Bay, the ecologic impact of such restoration, particularly in aquaculture settings, has largely been overlooked. As filter feeders, oysters have a major impact on the water column by moving phytoplankton and suspended sediment to the benthos, otherwise referred to as benthic-pelagic coupling. Since oysters have the ability to continuously filter the water around them, they also continuously excrete remineralized nutrients while using oxygen in the process.

This study will look to assess the impact on water quality by a commercial oyster aquaculture facility in the Chesapeake Bay, along with the potential for integration of the Algal Turf Scrubber – an ecologically engineered system designed to clean eutrophic waters through controlled algal production – with the commercial oyster aquaculture facility.

1.2 Background

1.2.1 History of Crassostrea virginica *in the Chesapeake Bay:*

The cultural eutrophication of the Chesapeake Bay is a result of both increased nutrient loading and the large decline in oyster biomass over the past century (Hagy et al., 2004; Kemp et al., 2005; Newell, 1988; Rothschild et al., 1994). Since the settlement by Europeans in the early 1600's, the declining water quality of the Chesapeake Bay caused by nutrient overloading in turn caused a shift from benthic primary production to planktonic primary production as the dominant process (Jackson et al., 2001; Cooper and Brush, 1993). This change has impacted the health of commercially valuable species, and overharvesting of some of these species has further impacted the health of the Bay (Kirby 2004; Officer et al., 1984).

Oyster harvest rates parallel this decline in Bay water quality, with the peak Chesapeake Bay oyster harvest occurring in 1884, a few years after the legalization of dredge harvesting (Rothschild, 1994; Heral et al., 1990). Total harvest of *C. virginica* in the Chesapeake Bay declined further with the discovery and persistence of the diseases *Dermocystidium marinum* in 1950, and *Minchinia nelsoni* (MSX) in 1959 (Andrews, 1979; Rothschild et al, 1994; Ewart and Ford, 1993; Ray, 1954; Burreson and Ford, 2004). Both diseases result in high oyster mortality rates, and have been linked to increased salinity levels (Hofmann et al., 2001; Ford, 1996; Cook et al., 1998), such as those after an extended drought when salinity increases above normal levels upwards into the Chesapeake Bay. The devastation caused by these diseases led to the discussion of potentially introducing disease-resistant, non-native oysters to the Chesapeake Bay

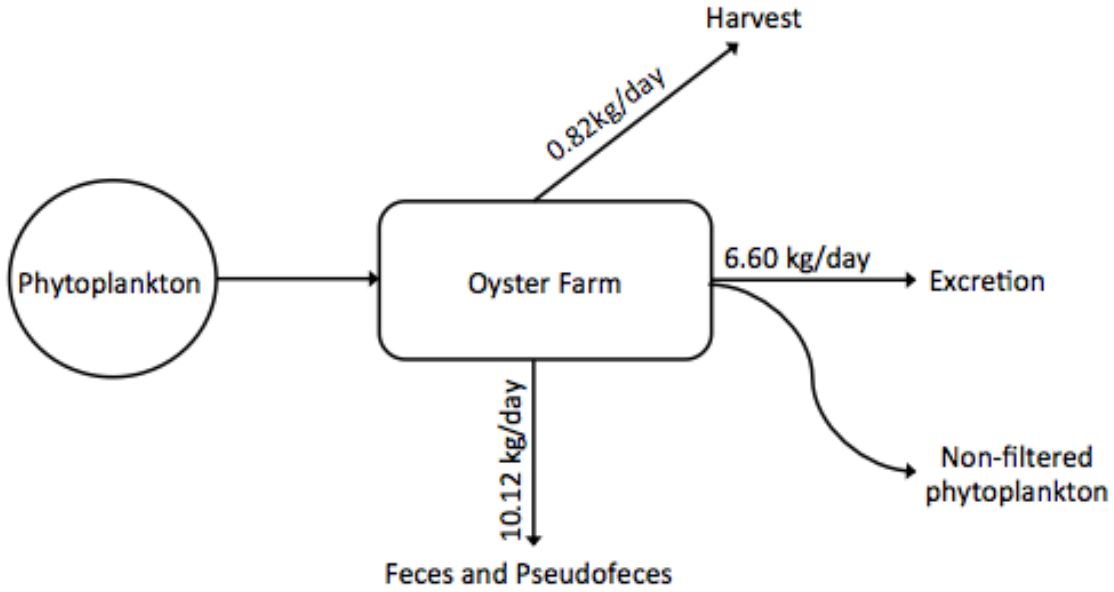
(Gottlieb and Schweighofer, 1996; Mann et al., 1991; Lipton et al., 1992), and the breeding of disease resistant *C. virginica* (Calvo et al., 2003).

Oyster harvests and bar locations have long been monitored in Maryland, with laws governing harvest area and total catch dating back to the 1800's (Kennedy and Breisch, 1983). More recently, there has been a political movement towards commercial oyster aquaculture facilities in the Chesapeake Bay, with a streamlined leasing process and tax incentives for owners (Maryland Oyster Advisory Commission, 2008).

1.2.2 Role of Crassostrea virginica *in the Nitrogen and Phosphorus Cycles:*

Eastern oysters play a critical role in the aquatic nitrogen cycle through benthic-pelagic coupling processes (Newell et al., 2005). *Crassostrea virginica* is a suspension-feeding bivalve, meaning it filters suspended particles from the water column, preferentially ingesting some particles while rejecting others as pseudofeces (Galtsoff, 1964; Haven and Morales-Alamo, 1970; Newell and Jordan, 1983; Ward et al, 1994; Newell, 2005). The main processes to be considered when discussing the role of *C. virginica* in the nitrogen and phosphorus cycles are: the filtration of sediment and phytoplankton; the process of feces and pseudofeces deposition; excretion of nitrogen and phosphorus by the oysters; and the incorporation of nitrogen and phosphorus into the oyster shell and tissue (Figure 1.1).

A. Nitrogen



B. Phosphorus

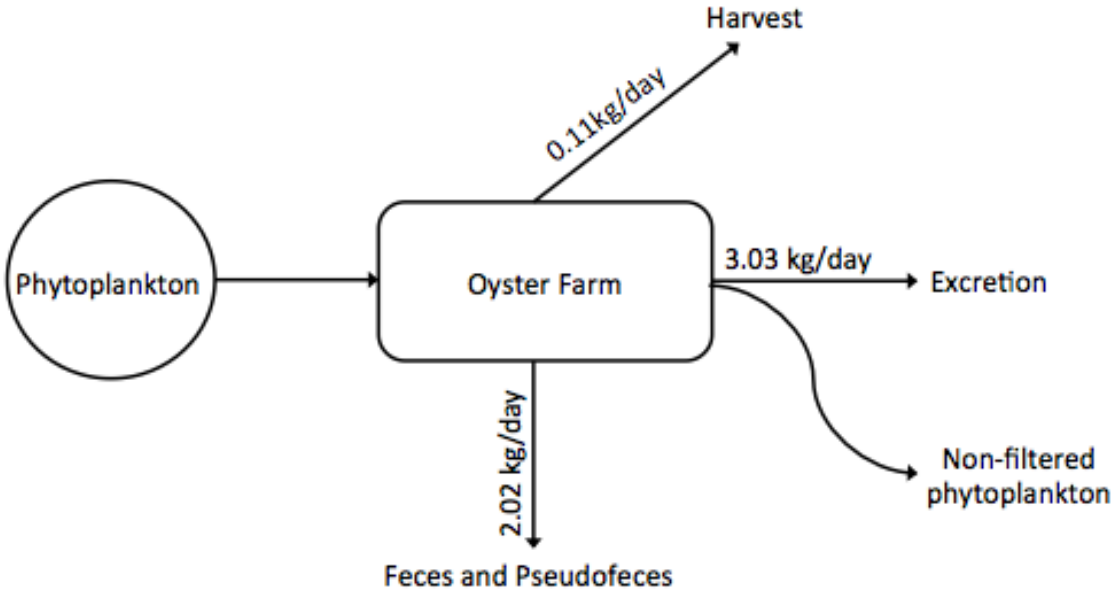


Figure 1.1: Modeled daily values of nitrogen (A) and phosphorus (B) fluxes by Marinetics oyster farm, assuming high concentrations of phytoplankton. Values were calculated from literature and data collected at Marinetics. Denitrifcation is ignored.

As a filter feeder, *C. virginica* removes small particles from the water column and either ingests or releases them. The filter feeding process of *C. virginica* and other bivalves is considered to be an important process in regulating phytoplankton population and turbidity (Caraco et al., 1997; Newell, 1988; Grabowski and Peterson, 2007; Coen et al., 2007). It has been suggested that the loss of the oyster population and this filtering capacity is one of the primary causes for decline in the health of the Chesapeake Bay. Newell and Jordan (1983) showed that *C. virginica* is able to preferentially reject or ingest items it filters. They found that chlorophyll-a levels – a measure of phytoplankton standing stock – were lower in pseudofeces than the ingested water, demonstrating consumption by the oyster. This reduction in chlorophyll-a available in the water column is associated with a reduced phytoplankton population, which often coincides with decreased turbidity. Assessments of chlorophyll-a levels both upstream and downstream of oyster beds in tidal creeks demonstrated the ability of *C. virginica* to remove large quantities of phytoplankton from the water column (Nelson et al., 2004; Wetz et al., 2002).

As oysters filter particles from the water column, they are either ingested or released as pseudofeces. These pseudofeces are made up of rejected phytoplankton, along with clay, silt and organic molecules (Haven and Morales-Alamo, 1966, 1968, 1970; Newell et al., 2005). Pseudofeces fall rapidly to the sediment, and become incorporated into the sediment, or broken apart and reintroduced into the water column. Biodeposition of pseudofeces has been estimated at rates of up to 981kg per week for .405 hectares of oyster covered estuary bottom (Haven and Morales-Alamo, 1966). Multiple studies have concluded that pseudofeces accumulation and the resulting nitrogen

and phosphorus regeneration from the sediment causes increased primary production in the areas of accumulation (Asmus and Asmus, 1991; Dame and Libes, 1993; Dame et al., 1989, 1992). However, it has also been suggested that through incorporation into the sediment and denitrification processes, pseudofeces production by *C. virginica* could potentially play a large role in the removal of both nitrogen and phosphorus from the water column (Newell et al., 2005). It is important to remember that *C. virginica* does not introduce any new nitrogen or phosphorus into the system, but regenerates it in a different chemical form. Nitrogen compounds are mostly organic in phytoplankton, and mostly inorganic in oyster excreta and biodeposits. At steady state, the nitrogen in is equal to the nitrogen out.

Crassostrea virginica also constantly excretes nitrogen and phosphorus. Nitrogen is excreted as ammonia, nitrate, nitrite, urea, and amino acids; it is excreted at different rates at different temperatures, with higher rates of excretion during warmer seasons, and decreased rates of excretion during cooler weather (Hammen, 1968, 1969; Srna and Baggaley, 1976; Pietros and Rice, 2002; Dame et al., 1992). Multiple studies have investigated rates of inorganic nitrogen excretion by *C. virginica* - most finding different rates of excretion - with the results from selected studies summarized in Table 1.1. Phosphorus is similarly excreted by *C. virginica*, but at lower levels not normally much above background levels (Dame et al., 1991; Asmus and Asmus, 1991).

Crassostrea virginica has a relatively high stress tolerance to ammonia, nitrate, nitrite, and orthophosphate (Epifanio and Srna, 1975). Ammonia may also serve as a chemical cue for oyster larvae when selecting a site to settle (Fitt and Coon, 1992). In the same study, Fitt and Coon also suggest that rates of ammonia excretion are not high

enough to trigger the settlement by these larvae, but the release of ammonia from sediment with high levels of pseudofeces deposition is. Kirby and Miller (2004) found that *C. virginica* grows more rapidly as a result of anthropogenic eutrophication due to more readily available phytoplankton (these same eutrophic conditions can cause oyster death through lack of available dissolved oxygen). These studies all suggest that *C. virginica* is adapted to, and may even prefer environments of high nitrogen and phosphorus content.

Table 1.1: Reported rates of ammonia excretion by *Crassostrea virginica* from previous studies. Units converted to µM/g dry weight day^{-1} for comparison.

Study	Reported Ammonia Excretion Rate	Converted Excretion Rate (µM/g dry weight day^{-1})
Srna and Baggaley (1976) Laboratory Study	25 µM/g day^{-1} in g dry tissue weight [a]	25
Hammen (1968) Laboratory Study	.298 - .978 µM/g day^{-1} in g wet tissue weight [b]	11.678 - 38.328
Pietros and Rice (2002) Mesocosm Study	$E = 50.65W^{.699}$ E: µgN/hr g^{-1} W: g dry tissue weight [a]	67.39
Dame et al. (1992) Field Study	125 gN/m^2 yr^{-1} 196g dry tissue per m^2 40% of ammonia from oysters [c]	38.75
Average[d]		**39.04**

[a], Assuming a 1 g dry tissue weight oyster
[b], Wet/dry tissue conversion rate of y = 84.29x – 45.1, where y is wet tissue and x is dry tissue; from Hammen (1968)
[c], From Boucher and Boucher – Rodoni, 1988
[d] Used the mean value from Hammen's laboratory study

Incorporation of nutrients into oyster tissue and shell is the fourth main component of *C. virginica's* influence on the nitrogen and phosphorus cycles. Many studies have investigated the chemical composition of the *C. virginica* shell and soft

tissue (Smith and Wright, 1962; His and Maurer, 1988; Simkiss, 1965; Yoon et al., 2003; Higgins et al., 2011; Grizzle and Ward, 2011). Most recently, Higgins et al. (2011) determined the percent composition on nitrogen and phosphorus of *C. virginica* from an aquaculture facility in the Chesapeake Bay. They determined nitrogen constitutes 7.9% tissue dry weight and 0.2% of shell dry weight. Phosphorus made up 0.8% of tissue dry weight and 0.04% of shell dry weight. An average aquacultured oyster would remove 0.13g of nitrogen and 0.02 g of phosphorus from the water column upon harvest. This demonstrates that a large-scale aquaculture facility harvesting a few hundred thousand oysters annually removes a significant amount of both nitrogen and phosphorus from the Chesapeake Bay through the harvest process alone. In the case of the facility being investigated in this study, Marinetics, it can be estimated that the annual harvest removes approximately 130kg of nitrogen, and 20kg of phosphorus.

1.2.3 Influence of Crassostrea virginica *on the Surrounding Ecological Community:*

There are two main methods by which a *C. virginica* reef or aquaculture facility can influence the surrounding ecological community: chemically and physically. Chemically, the oysters alter the ratio and availability of nutrients, particularly nitrogen and phosphorus as mentioned in the preceding section; physically, the oyster reef alters local water flow dynamics, increases the amount of available hard surface area for sessile species, and provides refuge for prey species.

Redfield discussed the importance of nutrient availability and ratios for aquatic productivity in his paper titled “The Biological Control of Chemical Factors in the

Environment" (Redfield, 1958). Since Redfield's seminal work, many studies have focused on the importance of nutrient ratios and availability in the aquatic environment on productivity and species composition. Specifically, several previous studies have investigated the chemical role of intertidal invertebrates on the macrofloral and the microbial community (Pfister, 2007; Bracken, 2004; Bracken and Nielsen, 2004; Schindler et al., 2004). The study by Pfister compared tidal pools with and without the mussel *Mytilus californianus,* and found that pools with mussels showed a 300% increase in biomass of the red macroalga *Prionitis lanceolata* when compared to pools with no mussels. This same study also found an increase in benthic microalgal abundance in pools with mussels as compared to those without, demonstrating the importance of regenerated N in algal production. Schindler et al. (2004) found that trout reintroduced to Rocky Mountain lakes altered the cycling of phosphorus and promoted algal productivity.

Nutrient stoichiometry, or the specific ratio of nutrients available in an ecosystem, influences microalgal population dynamics and species growth rates (Altman and Paerl, 2012; Piehler et al, 2004; Li et al., 2012; Gobler et al., 2012; Glibert, 2012). Altman and Paerl showed that different available forms of nitrogen promoted growth of different microalgal groups (dinoflagellates, chlorophytes, cyanobacteria, etc). Terlizzi and Glibert (1999) found dinoflagellate blooms to co-occur with elevated concentrations of urea in a finfish aquaculture facility. Other studies have shown that diatoms grow rapidly when nitrate is the most available form of N, and dinoflagellate growth has been found to correspond with high levels of ammonia (Berg et al., 2003; Harrison and Turpin, 1982; Paerl, 1988). Few, if any studies have investigated the influence of a ratio of nutrients as excreted by oysters on phytoplankton community structure. As oysters excrete high

levels of ammonia, it may be expected that they support growth of dinoflagellate populations downstream of reefs, or aquaculture facilities.

Oyster reefs increase local fish productivity and biomass through providing refuge from predators for smaller fish, and in turn more available prey for predatory fish (Peterson et al., 2003; Meyer and Townsend, 2000; Rodney and Paynter, 2006; Francis et al., 1999). Physical structure of oyster reefs can also provide the hard substrate needed for other species, which in turn also act to clean the water, such as tunicates (Ulanowicz and Tuttle, 1992). Recent studies comparing species diversity and richness in natural oyster reefs, oyster aquaculture facilities/reefs, and non-vegetated seabed all indicate that aquaculture facilities and equipment provide similar or superior habitat than the natural reefs (Erbland and Ozbay, 2008; Marenghi et al., 2010; Dealteris et al., 2004). Both the natural and aquaculture reefs were of a superior habitat value to the non-vegetated seabed.

1.2.4 The Algal Turf Scrubber and Integrated Multi-Trophic Aquaculture

The Algal Turf Scrubber™ (ATS) is an ecologically engineered water treatment system designed to remove large quantities of inorganic nutrients from a water body along with producing a harvestable algal biomass (Adey et al., 1993, 2011; Kangas and Mulbry, 2014; Mulbry et al., 2010). The ATS system consists of an attached algal community growing on screens in a shallow water trough or raceway through which water is pumped. As water is pumped onto the raceway, the algal community provides water treatment by uptake of nutrients during photosynthesis. Algae remove the nutrients

through biological uptake and "capture" them through biomass production. At the end of the raceway, water is released back into the waterway, with a lower nutrient concentration than when it was pumped into the system. Algae are harvested approximately once per week during the ATS growing season, effectively removing the captured nutrients in the form of algal biomass. Because of the fast growth rate of algae on the ATS, this technology can remove nutrients from water at a rapid rate. Harvesting is also important as it rejuvenates the algal community and helps to maintain high growth rates.

Previously, experiments with the ATS have been focused on mitigating nonpoint source pollution and animal waste (Table 1.2). While this may be the first study investigating the potential for integrating the ATS with bivalve aquaculture, previous studies investigating the potential for large scale algal production using integrated multi-trophic aquaculture (IMTA) have shown promise for high productivity rates, nutrient removal, and the production of a marketable product (Neori et al., 2004). The theory behind IMTA is to mimic nature and incorporate organisms from different trophic levels in order to maximize biomass production and minimize potentially toxic levels of nitrogen (specifically ammonia) in a specified area. Finfish, shellfish, and algal culture are operated together, with the bivalves consuming finfish waste and phytoplankton produced by excreted nutrients. The macroalgae serves to absorb excess nutrients and maintain levels of dissolved oxygen in the IMTA facility through photosynthesis. Currently, most IMTA facilities and studies use either rope-cultured algae, or algal filled ponds. The hypothetical changes in water quality through integration of the ATS with oyster aquaculture can be seen in Figure 1.2.

Table1.2: Summary of select previous studies using ATS.

Study	Type of Wastewater Treated	Average Growth Rate ($g/m^2\ day^{-1}$)	Tissue N Content (gN/100g tissue)	Tissue P Content (gP/100g tissue)
Adey and Goertemiller (1987)	Original study of screens in a coral reef	14	n/a	n/a
Kebede-Westhead et al. (2006)	Swine manure	9.4	5.7	1.8
Mulbry et al.(2008)	Dairy manure	2.5 (low loading rate) 25 (high loading rate)	4.3 7.0	.64 .91
Mulbry and Wilkie (2001)	Dairy manure	5	5 – 7	1.5 – 2
Wilkie and Mulbry (2002)	Dairy manure	5.3 (low loading rate) 5.5 (high loading rate)	4.9 7.1	1.5 2.1
Kebede-Westhead et al. (2003)	Anaerobically digested dairy manure	4.9 (low loading rate) 21.8 (high loading rate)	2.9 7.3	.5 1.3
Craggs et al.(1996)	Secondary sewage effluent	35	n/a	2.1
Adey et al. (1993)	Agricultural canal (Florida Everglades)	15-39 [1]	n/a	.34 – .43
Mulbry et al (2010)	Chesapeake Bay tributaries:			
	Patuxent	1.2 – 2.9 [1]	.8 – 2.0	.20 – .34
	Bush	.01 – 10.4 [2]	.9 – 3.2	.22 – .36
	Patapsco	.5 – 7.5 (Winter only)	1.6 – 2.9	.14 – .36
Kangas (2011)	Heated discharge waters from a nuclear power plant	7.1 – 21.6 [2]	n/a	n/a
Kangas et al. (2009)	Chesapeake Bay tributary: Susquehanna	14 (aluminum ATS) [1] 11.7 (wood ATS)	2 – 3	.25 – .35
Kangas and Mulbry (2014)	Agricultural drainage ditch (Maryland Eastern Shore)	2.8 – 12.6[2]	n/a	n/a
May et al. (2013)	Urban harbor (Baltimore, MD)	7.1 – 7.8	3.2	0.05

[1]Rates measured through a whole year

[2]Rates measured during growing season (March – August/September) only

Low NH_4^+ Concentration
Low PO_4^{3-} Concentration
High Dissolved Oxygen Level
High Chlorophyll-a Level

Increased NH_4^+ Concentration
Increased PO_4^{3-} Concentration
Decreased Dissolved Oxygen Level
Decreased Chlorophyll-a Level

High NH_4^+ Concentration
High PO_4^{3-} Concentration
Low Dissolved Oxygen Level
Low pH Level

Decreased NH_4^+ Concentration
Decreased PO_4^{3-} Concentration
Increased Dissolved Oxygen Level
Increased pH Level

Low NH_4^+ Concentration
Low PO_4^{3-} Concentration
Low pH Level
Average Dissolved Oxygen Level
High Chlorophyll-a Level

Decreased NH_4^+ Concentration
Decreased PO_4^{3-} Concentration
Increased pH Level
Increased Dissolved Oxygen Level
Decreased Chlorophyll-a Level

Figure 1.2: Hypothetical changes in water quality parameters in an Oyster Aquaculture facility, an ATS, and an integrated Oyster Aquaculture – ATS Facility.

1.3 Comparison of N Cycle in a River Segment with Oysters, an ATS, and IMTA

Figures 1.3 and 1.4 show the flow of N through a segment of river with and without oysters. In Figure 1.3 (river segment with no oysters) the main N pathways are the flow of plankton through the river segment, atmospheric deposition, denitrification, and fluxes between sediment and the water column. There is a small quantity of planktonic N moved to the sediment through deposition.

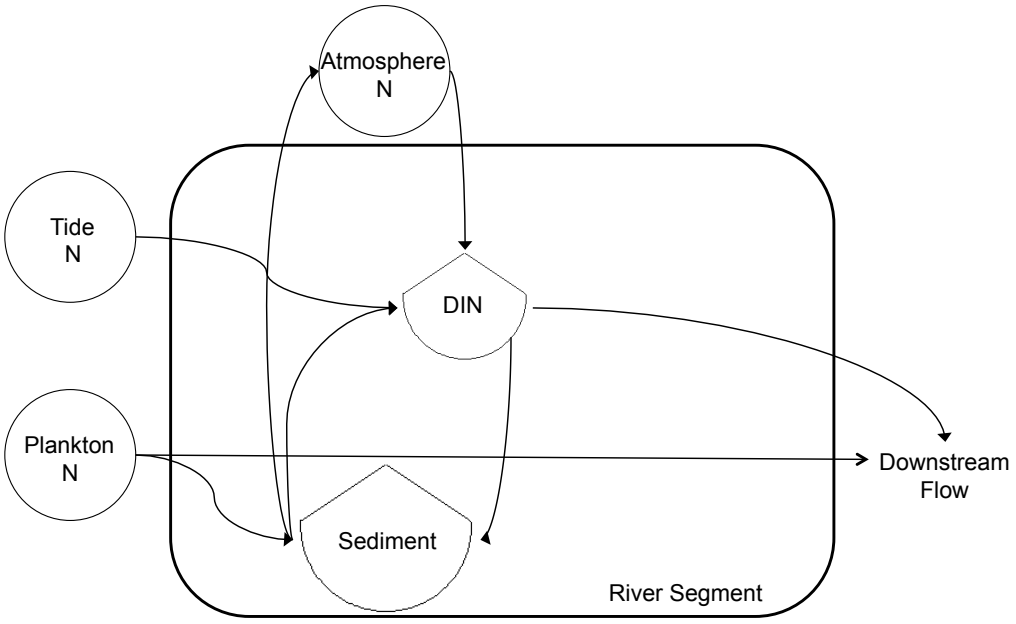


Figure 1.3: N cycle for a section of river.

When oysters are added to the river system (Figure 1.4), four additional N pathways must be included: filtration of plankton by the oyster, deposition of feces and pseudofeces to the sediment, excretion of DIN, and harvest of the oysters. The flow of N from the sediment to the atmosphere would increases as a result of increased denitrification from deposition of oyster feces and pseudofeces. There would also be an increased flow of DIN downstream to correspond with a decreased total downstream flow

of planktonic N. Overall, the addition of oysters to the river segment would shift a portion N flow from downstream flow to harvest and atmospheric storage.

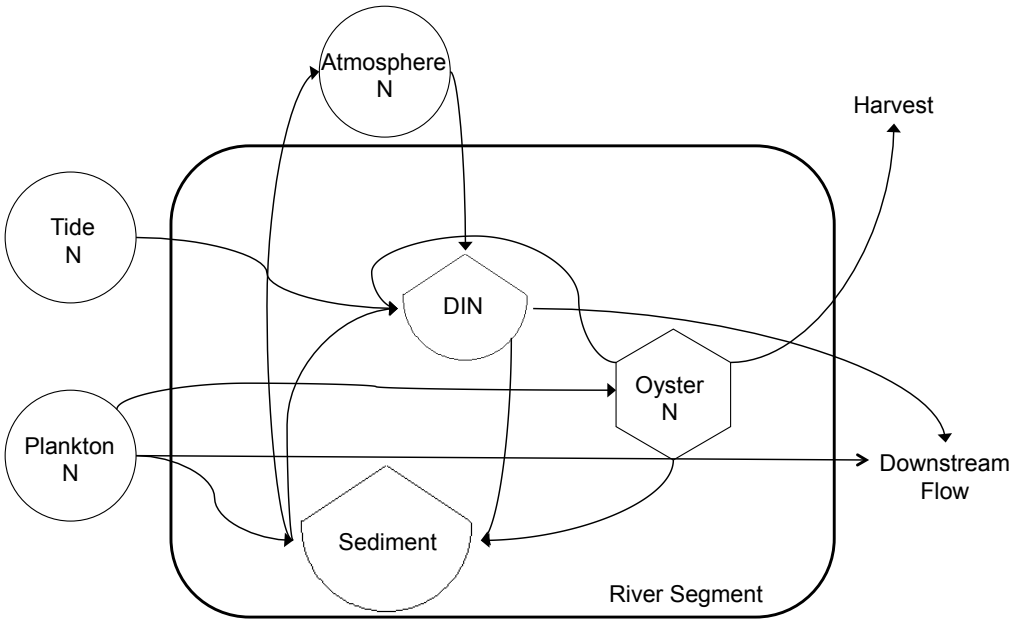


Figure 1.4: N cycle for a section of river with oysters.

When an ATS is added to a river segment (Figure 1.5), there are three more N pathways to be considered. The largest two pathways are the uptake of DIN by the production of algae in the ATS, along with the corresponding harvest. The third pathway is plankton N being deposited in the ATS. Downstream flow of DIN should decrease significantly with the addition of an ATS to the system.

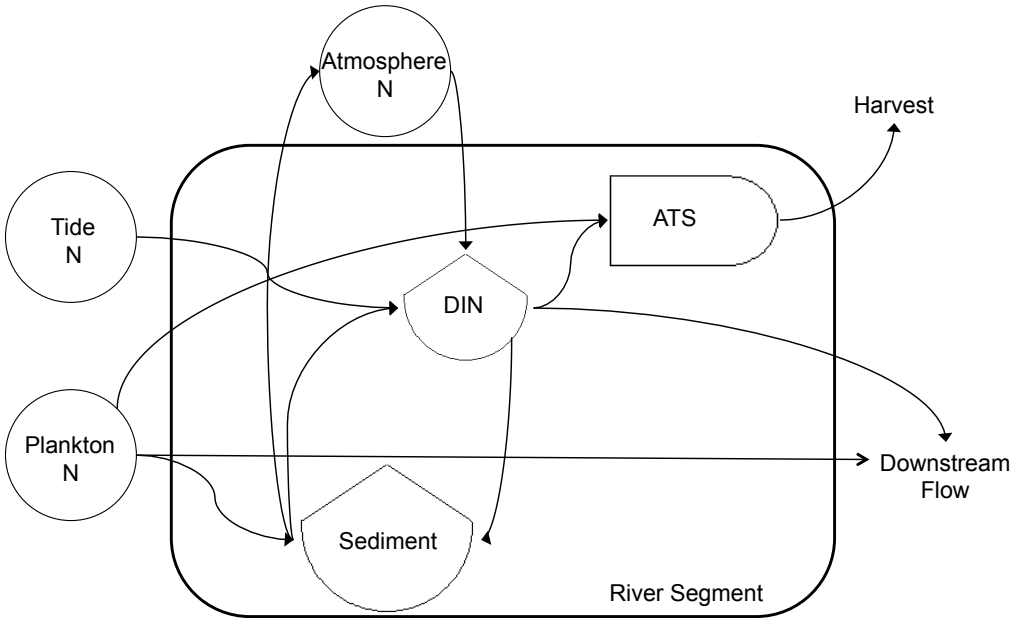


Figure 1.5: N cycle for a section of river with an ATS.

When both an ATS and oysters are present in the form of IMTA in a section of river (Figure 1.6), only one additional N pathway is added when compared to a river segment with an ATS, or a river segment with oysters. This flow is the capture of oyster excretion by the algal growth in the ATS. In a river segment with IMTA, it can be expected that the dominant flows of N out of the system will be through harvest of the oysters and ATS and through an increased rate of denitrification from the sediment to the atmosphere. These increases will correspond with a significant decrease in the downstream flow of both DIN and N in plankton.

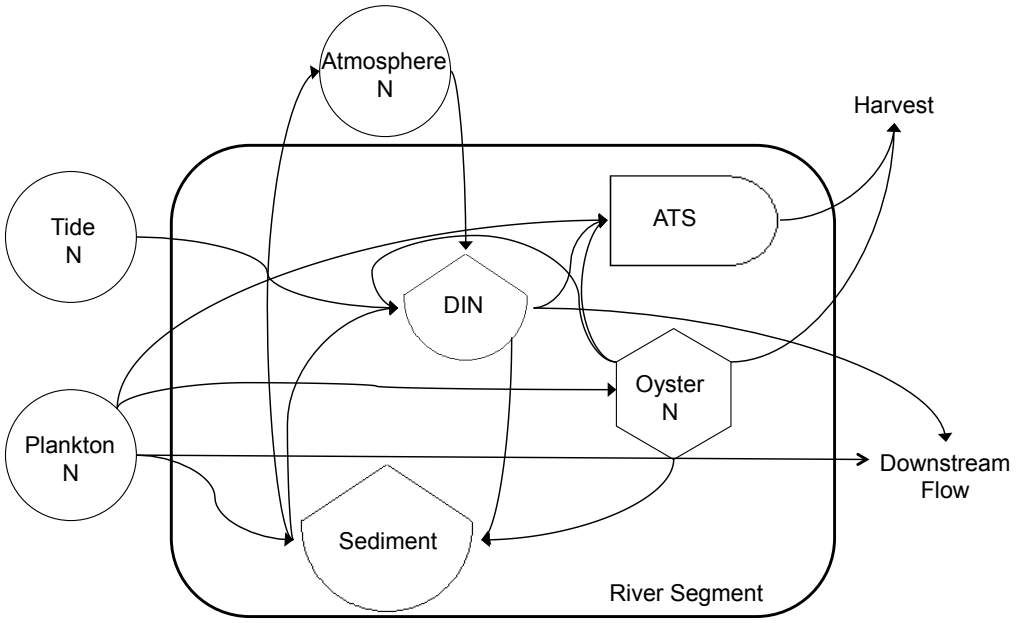


Figure 1.6: N cycle for a section of river with oyster-ATS IMTA.

1.4 Hypotheses

The following hypotheses were proposed for the study:

- Hybrid triploid oysters grown at the aquaculture facility will excrete ammonia at a comparable rate to past studies using diploid oysters.
- Water will have a higher concentration of ammonia after passing through the oyster aquaculture facility.
- Water will have a higher concentration of phosphates after passing through the oyster aquaculture facility.
- Water will have no change in nitrate concentration after passing through the oyster aquaculture facility.
- Water will have no change in nitrite concentration after passing through the oyster aquaculture facility.
- There will be no change in temperature or salinity as water passes through the oyster aquaculture facility.
- Water will have a lower concentration of chlorophyll-a after passing through the oyster aquaculture facility.
- Water will have a lower concentration of dissolved oxygen after passing through the oyster aquaculture facility.
- Increased rates of water flow through the facility will be associated with smaller changes in water quality parameters.
- An Algal Turf Scrubber installed at the oyster aquaculture facility will have a higher growth rate than those installed at other locations without aquaculture.

CHAPTER II: Methods

2.1 Site Description

Fieldwork was conducted at Marinetics Inc., a commercial oyster aquaculture facility located in Lecompte Bay, near the mouth of the Choptank River on Maryland's Eastern Shore (Figure 2.1 and 2.2). The average water depth at the facility ranges from .5-1.5 meters. There is a dominant tidal influence and brackish water, with average salinities ranging from 10-15 ppt annually (Chesapeake Bay Program, 2012), and water temperatures ranging from 3 to 30ºC throughout the year (Maryland Department of Natural Resources, 2014). The facility employs raft aquaculture, using floating mesh cages on the surface of the water to grow the oysters. At any time there are between 8,000,000 – 10,000,000 oysters in the cages, with between 750,000 and 1,000,000 of them harvested annually. Each floating cage holds approximately 200-400 oysters. The surrounding land is predominantly used for agriculture.

Oysters raised at the facility are of the native species *Crassostrea virginica*, but are a genetically modified triploid hybrid, as opposed to the native diploid. Triploid oysters are market ready more quickly, and are more resistant to MSX and dermo. The oysters raised at Marinetics are typically purchased as spat, and then raised for two years at which point they are considered market size. Oysters from Marinetics are sold throughout the Chesapeake Bay region at Whole Foods grocery stores, and seafood restaurants.

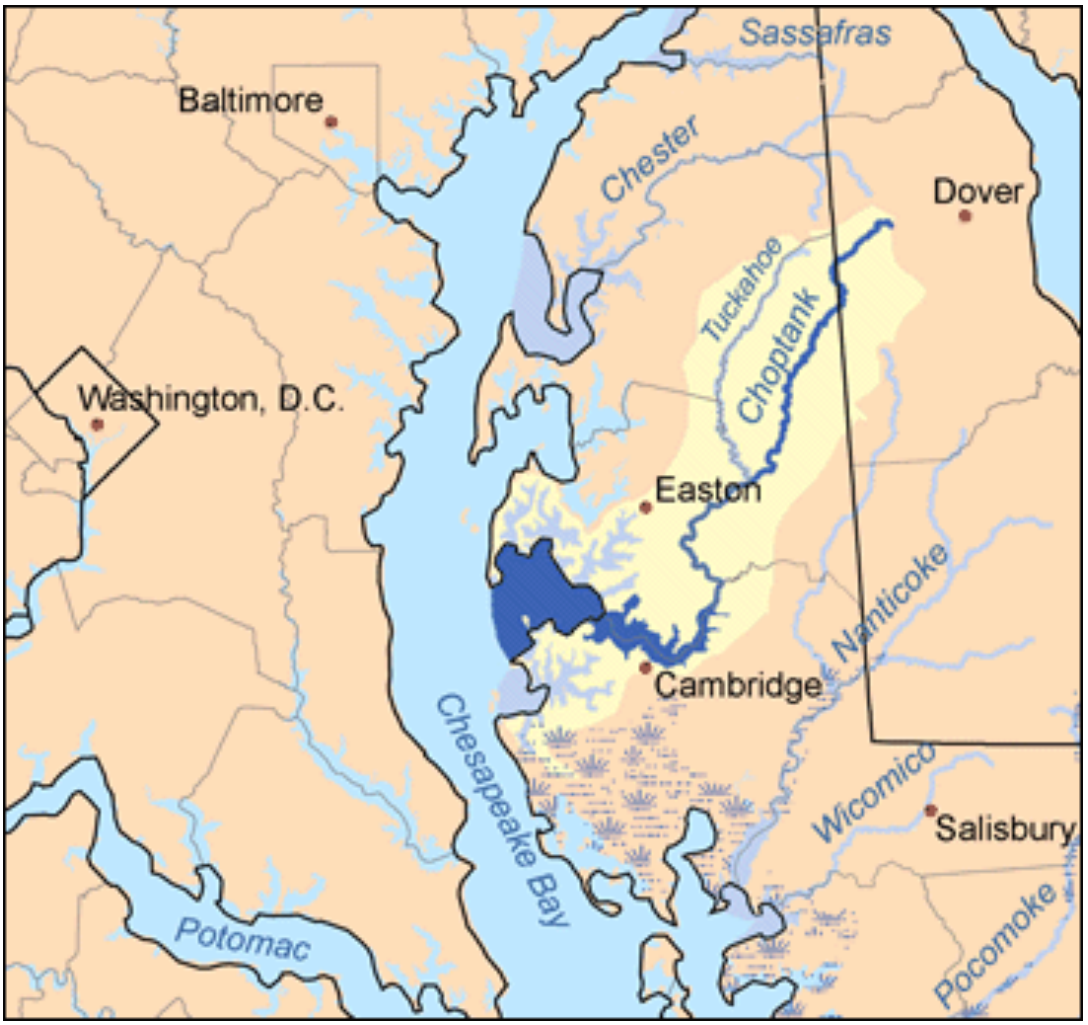


Figure 2.1: The study site is located approximately 8km west of Cambridge. Image from http://upload.wikimedia.org/wikipedia/commons/1/18/Choptankmap.png (assessed 3/14/2014)

Figure 2.2: Aerial view of Marinetics oyster farm. Flow of water is typically from South to North. The stars indicate sampling areas. Image from https://www.google.com/maps/@38.6257292,-76.168458,321m/data=!3m1!1e3 (assessed 1/25/2014)

2.2 Oyster Impacts

2.2.1 Oyster Excretion Experiments

To assess the excretion rates of the oysters at the aquaculture facility, a simple set of methods were developed. The experimental treatment (n=3) consisted of twelve oysters of approximately equal individual and total wet weight, taken from the aquaculture facility and placed in an 8L plastic tub containing 4-6L of water collected from the river near the aquaculture facility (river water was compared to DI in an earlier experiment, and no difference in excretion rate was evident, so river water was used for each sampling date). Oysters were allowed to acclimate in the tub for 30 minutes before sampling began. The first 30 ml water sample was collected at Time 0, and again every thirty minutes over a two-hour period. Samples were filtered through a glass fiber filter, then placed on ice and returned to the lab.

Samples were analyzed for ammonia using the ammonia salicylate method (APHA, 1980). Excretion rate was calculated as change in concentration divided by time. This experiment was performed four times: March 8, 2012; June 28, 2012; April 22, 2013, and July 16, 2013 (encompassing two cold weather dates, and two warm weather dates).

2.2.2 Upstream/Downstream Water Quality

The field assessment of potential impact on water quality was conducted from May through October 2013. As there is a tidal influence at the study site, direction of water flow through the facility was determined on each sampling date using a float

attached by a thread to a compass. The direction indicated by the thread was recorded at each of the three sampling points (indicated on Figure 2.2), and then compared against a map of the facility to ensure an accurate flow direction and determination of which point was "upstream" and which was "downstream". "Upstream" was water that had not entered the facility, or inflow, while "downstream" was water that had passed through the facility, or outflow. Flow speed was also measured at each sampling point using a Model 2100 Current Velocity Meter (Swoffer Instruments Inc., Seattle, Washington).

Dissolved oxygen, salinity, and temperature, were recorded on site at the upstream, middle, and downstream points using a YSI 55 handheld meter (YSI Inc., Yellow Springs, Ohio). Water samples (500ml) were collected at each sampling point. The collected samples were placed on ice and vacuum filtered through glass fiber filters (Whatman 47mm, 5.0μM pore size) upon return to the lab. When there was approximately 10ml of sample still to be filtered, already filtered water was removed and either frozen for analysis at a later date, or immediately analyzed for ammonia, nitrate, nitrite, and phosphates using techniques described below. The water still in the filter had three drops of 1% $MgCO_3$ suspension added so that the filters could be assessed for chlorophyll-a concentration, as described by Yentsch and Menzel (1967).

After filtration, filters were stored in labeled centrifuge tubes and frozen for further analysis. All analyses for chlorophyll-a were conducted within a month of freezing the filter. Upon removal from the freezer, filters were ground with 5ml of 90% acetone using a tissue grinder. The ground filter/acetone mix was then returned to the centrifuge tube and placed in a 4°C refrigerator for 6 hours. After removal from the refrigerator, the mixture was centrifuged for 20 minutes at 2000rpm. One milliliter of

supernatant was taken from the centrifuge tube using a pipette and moved to a spectrophotometer cuvette with a light pathway of 10mm. Absorbance was measured at λ=750, 663, 645, and 630 using a spectrophotometer. Chlorophyll-a concentration in μg/L was calculated by the following equation:

$$Chlorophyll - a\ \left({}^{\mu g}/_{L}\right) = \frac{[11.64(\lambda 663 - \lambda 750) - 2.16(\lambda 645 - \lambda 750) + 0.10(\lambda 630 - \lambda 750)] \times acetone\ volume(mL) \times .90}{volume\ sample\ filtered(L) \times cell\ path\ length\ (cm)}$$

The filtered water samples were analyzed for ammonia using the ammonia salicylate method, nitrate by the cadmium reduction method, nitrite by the diazotization method, and phosphate by the ascorbic acid method (APHA, 1980).

Laboratory analyses were conducted at the Institute of Marine and Environmental Technology in Baltimore, Maryland, and at the University of Maryland in College Park, Maryland.

2.2.3 Creating a Predictive Model for Nutrient Release

A simple plug flow model for predicting the change in concentration of ammonia from upstream to downstream of the facility was developed for comparison with field-collected data. Variables in the model include rate of flow through the facility, number of oysters present, temperature, and volume. The model can be seen in the following equation:

$$Change\ in\ Concentration = \frac{Rate\ of\ Excretion \times\ Number\ of\ Oysters \times Volume}{Area \times Flow\ Rate}$$

Or:

$$\Delta \mu M\ NH_4^+ = \frac{\mu M\ NH_4^+ / Oyster * Day^{-1} \times Number\ of\ Oysters \times m^3}{m^2 \times {}^{m}/_{s} \times {}^{24hr}/_{day} \times {}^{60min}/_{hr} \times {}^{60s}/_{min}}$$

The model was run using two different ammonia excretion rate values. The first used the average warm weather excretion rate determined in the oyster excretion studies described previously (45.08 μmolNH_3/g dryweight*day^{-1}), and the second variable used the average value from the previous studies (39.04 μmolNH_3/g dryweight*day^{-1}).

Rate of flow of water through the facility was recorded for most sampling dates, with an average flow rate of 0.04 m/s throughout summer 2013. The area covered by oyster floats at the facility was approximately 5850m^2 (45m from east to west, 130m from north to south), with approximately 8,000,000-10,000,000 oysters in that area (for the model, the 10,000,000 value was used). The volume of water moving through the facility was estimated by multiplying the area covered by the oyster cages with an estimated "depth" of 0.1m. This value was chosen through the assumption there was minimal vertical mixing in the water column at the facility, and the maximum depth below water surface of the oyster floats was 0.1m (most floats were no more than 0.05m below the water surface). The calculated volume of water for this model is 585m^3.

The resulting values derived from the model were then divided by 45,000, the relative area of the opening of the sample bottle compared to the cross sectional area of the facility, for comparison with field-collected data.

On dates where the measured flow rate was below detection, a value of 0.005 was assigned for use in the model. This value was selected, as there was some visible flow, but not enough to spin the propeller on the flow meter. The lowest value the flow meter was able to display was 0.01, so it was decided to use half of that value in order for the model to work (a value besides zero was necessary, as flow rate is multiplied in the denominator, and a zero value would result in an attempt to divide by zero).

2.2.4 Statistical Analyses

For the upstream/downstream study, a one-tailed, paired two-sample t-test was used to assess for significant differences of each parameter from upstream to downstream through the summer. Results of the t-test were considered significant when $p < 0.05$.

In an attempt to further understand the influence of flow rate through the facility on change in parameter concentration, linear regressions were used. The difference from upstream to downstream of each parameter that had a statistically significant change was compared against the flow rate of water through the facility, and a coefficient of determination (R^2) was calculated. Regressions were also used in an attempt to find a relationship between upstream Chl-a concentration and the change in concentration of Chl-a from upstream to downstream. Change in ammonia concentration was also compared against the upstream concentration of Chl-a.

To compare the model against field data, a paired t-test was used. Field data was considered significantly different from the model if $p < 0.05$. All statistical tests were completed using StatPlus (AnalystSoft Inc., Alexandria VA).

2.3 Integration of the Algal Turf Scrubber

2.3.1 Operation of Algal Turf Scrubbers

Experimental-scale ATS were constructed of fiberglass troughs with a 1 m^2 screen growing area. The ATS were operated by pumping water from the aquaculture facility into a fiberglass dump-bucket, designed to tip into the trough and across the growing area every 8-10 seconds to simulate wave action. Drainage holes were made on the opposite side of the trough from the dump-bucket to allow adequate drainage and circulation of water in the ATS (Fig 2.3 and 2.4). The ATS were angled at a slope of approximately 1-2%. The water was pumped using a ½ hp Super Pump pool pump (Hayward Pool Products, Clemmons, NC, USA).

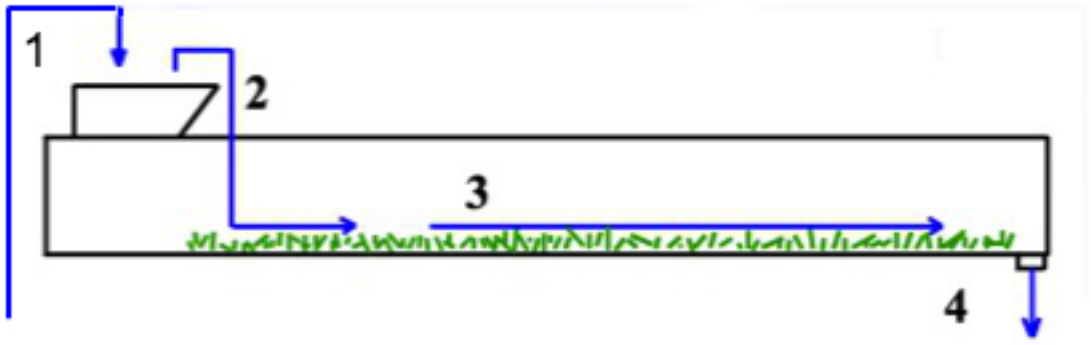


Figure 2.3: Simple schematic of the Algal Turf Scrubber; water from the oyster aquaculture facility is pumped into the dump bucket (Step 1). When the dump bucket is sufficiently full, it dumps out (Step 2), creating a small wave that flows across the attached algal turf where nutrients are "scrubbed" from the water and oxygen is added through photosynthesis (Step 3). When water reaches the end of the ATS flow way, it is drained back into the oyster aquaculture facility (Step 4).

The ATS were located on the facility dock, which is surrounded by oyster floats, and receives direct sunlight with little to no shading. One ATS was installed on September 10, 2012 and operated until October 22, 2012. Three ATS were installed May

13, 2013 and operated until October 25, 2013. Algal biomass and trapped sediment was harvested either weekly or biweekly using a wet/dry shop vacuum (Ridge Tool Company, Elyria, OH). The harvested algae was then dewatered by sieving it through 2mm mesh netting, then air dried at approximately 25°C for at least 48 hours to approximately 90% solids. For samples collected during Summer 2013, water from the sieving process was also collected ("green water" or GW), the volume was measured, and a subsample was taken. This subsample was then poured into an evaporating pan until 90% of the water had evaporated, leaving behind algal biomass and sediment that passed through the 2mm netting (all harvesting procedures adapted from Mulbry et al., 2010). Samples were dried at University of Maryland, College Park, and the Institute for Marine and Environmental Science (IMET) in Baltimore. At the time of each harvest, the most common algal taxa growing in the ATS were assessed and recorded. Common species growing at the aquaculture facility were also recorded.

A

B

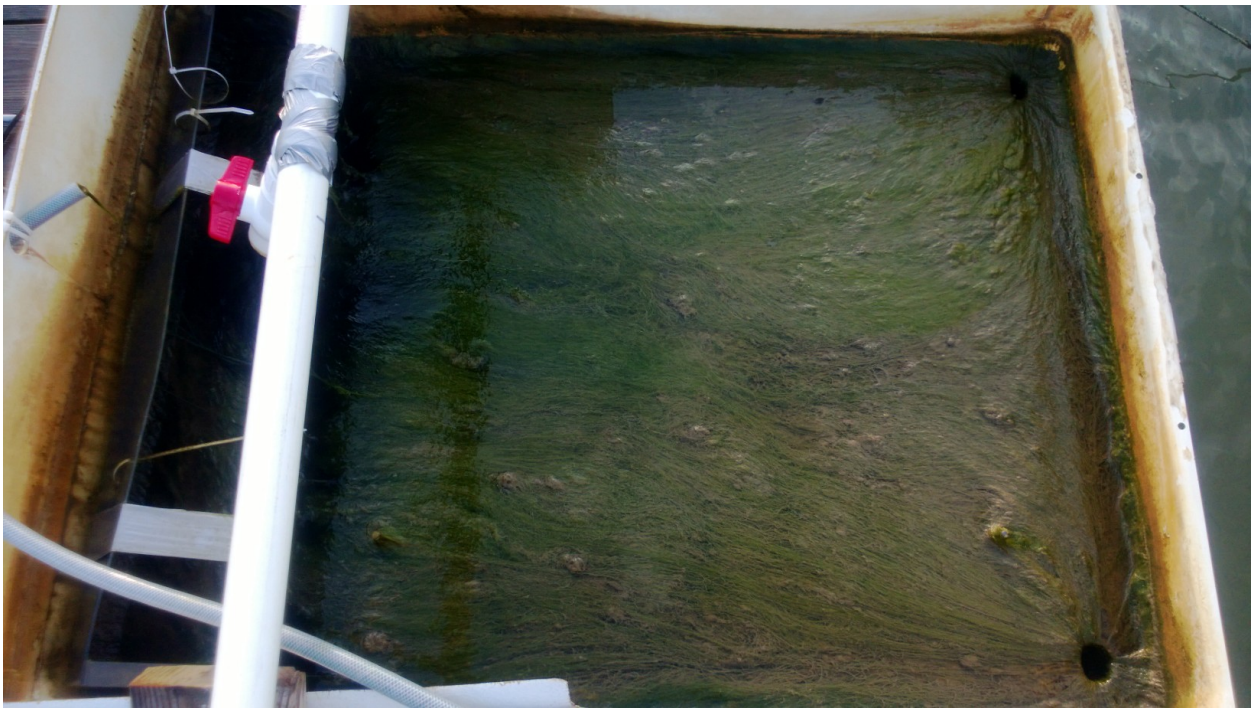


Figure 2.4: A) Three experimental ATS on the dock of the oyster aquaculture facility, with oyster floats in the background. B) ATS ready for harvest after one week of growth.

2.3.2 Harvested Algae Sample Preparation and Analysis:

After air-drying, samples were placed in a drying oven at 40°C for 48 hours, weighed, and then stored in sealed plastic bags. Subsamples were taken from each harvest date and finely ground using a coffee grinder. Samples from harvests between September 17, 2012 and October 22, 2013 were analyzed for total Kjeldahl nitrogen (TKN) using the Nessler method and total phosphorus (TP) using the ascorbic acid method following an acid persulfate digestion (APHA, 1980).

Another subsample was taken from 15 of the harvest dates (5 from Summer 2012 and 10 from Summer 2013) and 3 green water samples to determine total volatile solids through ashing. Samples were placed in a 70°C drying oven for at least 48 hours. Approximately 1g of sample was added to a porcelain crucible that had previously been burned in a muffle furnace at 550°C for one hour and then weighed. The mass of the sample and crucible was then recorded. The crucible containing the sample was then heated in a muffle furnace at 550°C for 1 hour to burn off any organic content. After combustion, the crucible and sample were weighed again. Dividing the loss in mass of the sample by the starting sample mass yielded the percent total volatile solids.

CHAPTER III: Results

3.1 Oyster Impacts

3.1.1Oyster Excretion

The average ammonia excretion rates ($mgNH_3/L*oyster^{-1}*hr^{-1}$) for each experimental date can be seen in Table 3.1. The average rate for all the experiments was 0.0215 $mgNH_3/L*oyster^{-1}*hr^{-1}$, which is equal to 30.29 $\mu molNH_3/g$ dryweight$*day^{-1}$. The average excretion rate for the two colder weather studies was 15.49 $\mu molNH_3/g$ dryweight$*day^{-1}$, and the average excretion rate for the two warm weather studies was 45.08 $\mu molNH_3/g$ dryweight$*day^{-1}$.

Table 3.1: Average ammonia excretion rate by oysters at Marinetics oyster aquaculture facility.

Date	**Average Excretion ($mgNH_3/L*oyster^{-1}*hr^{-1}$)**
3/8/12	0.014 ±0.00037
6/28/12	0.033 ±0.00042
4/22/13	0.008 ±0.00012
7/16/13	0.031 ±0.00037
Average	**0.0215 (or 30.29 $\mu molNH_3/g$ dryweight$*day^{-1}$)**

3.1.2 Upstream/Downstream Water Quality

Dissolved oxygen levels were higher upstream than downstream of the aquaculture facility on every sampling date except June 3 (Figure 3.1). Results of the paired t-test showed a statistically significant decrease in dissolved oxygen as water passed through the facility from upstream to downstream ($p = 0.0001$). The average upstream concentration of dissolved oxygen was 7.14 mg/L and the average downstream value was 6.66 mg/L.

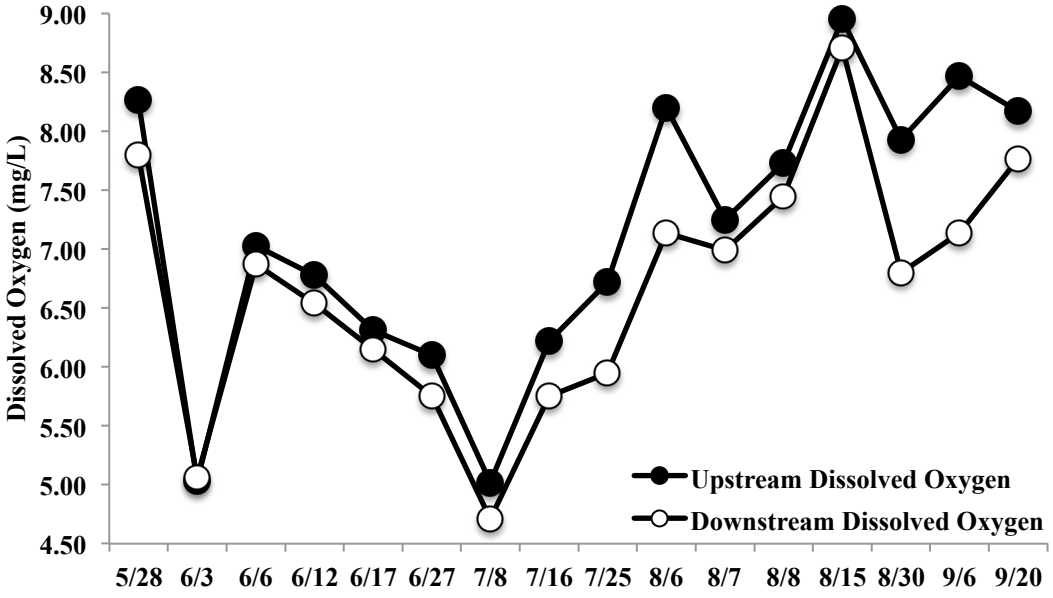


Figure 3.1: Upstream and downstream dissolved oxygen (mg/L) at Marinetics oyster farm during Summer 2013.

The regression to compare change in DO concentration with flow rate (Table 3.2) showed no significant correlation ($R^2 = 0.044$; Figure 3.2).

Table 3.2: Flow rate of water through Marinetics oyster aquaculture facility. For calculating averages for the predictive model, measurements below detection were given a value of 0.005 (represented in the table by dashes).

	Flow	Flow Rate (m/s)			
Date	**Direction**	**Upstream**	**Middle**	**Downstream**	**Average**
5/28/13	S to N	0.01	0.01	0.03	0.017
6/3/13	N to S	0.02	-	-	0.007
6/12/13	S to N	-	-	0.05	0.017
6/17/13	S to N	0.07	0.10	0.13	0.10
6/27/13	S to N	0.13	0.12	0.24	0.163
7/8/13	S to N	-	0.01	0.04	0.017
7/16/13	S to N	-	0.07	-	0.023
7/25/13	N to S	0.06	0.04	0.08	0.06
8/8/13	S to N	0.02	0.01	0.02	0.017
8/15/13	S to N	0.10	0.09	0.16	0.117
8/23/13	N to S	0.05	0.02	-	0.027
9/6/13	N to S	-	-	-	0.005
9/20/13	S to N	-	-	-	0.005
10/4/13	S to N	-	-	-	0.005
Average	-	0.033	0.034	0.054	**0.04**

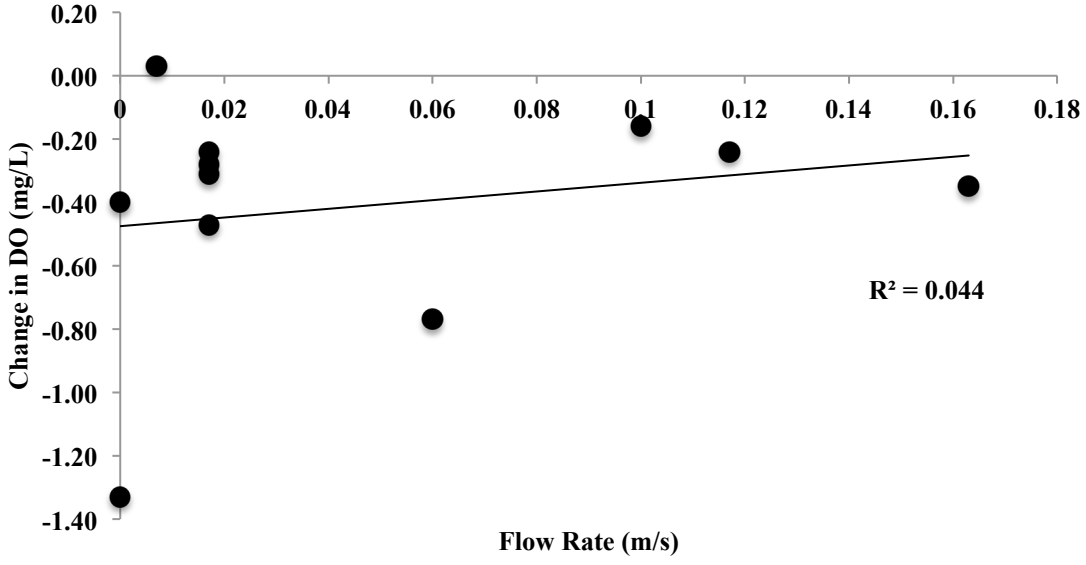


Figure 3.2: Comparison of flow rate (m/s) and change in DO concentration (mg/L) at the aquaculture facility in Summer 2013.

Salinity ranged from a low of 8.7 ppt on July 25 to a high of 12.3 ppt on September 20 (Figure 3.3). There was no significant difference in salinity from the upstream to downstream side of the facility ($p = 0.30$).

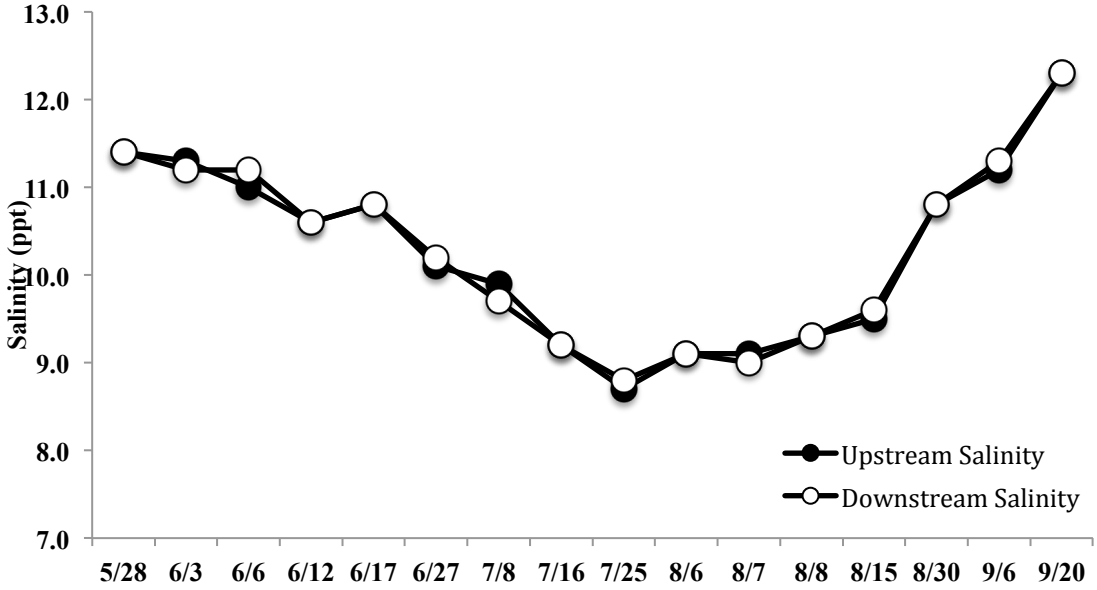


Figure 3.3: Upstream and downstream salinity (ppt) at Marinetics oyster farm during Summer 2013.

Temperature also did not vary significantly from upstream to downstream as water passed through the facility ($p = 0.1$). Temperature ranged from a low of 21.1 °C on May 28, to a high 30.3 °C on July 16 (Figure 3.4).

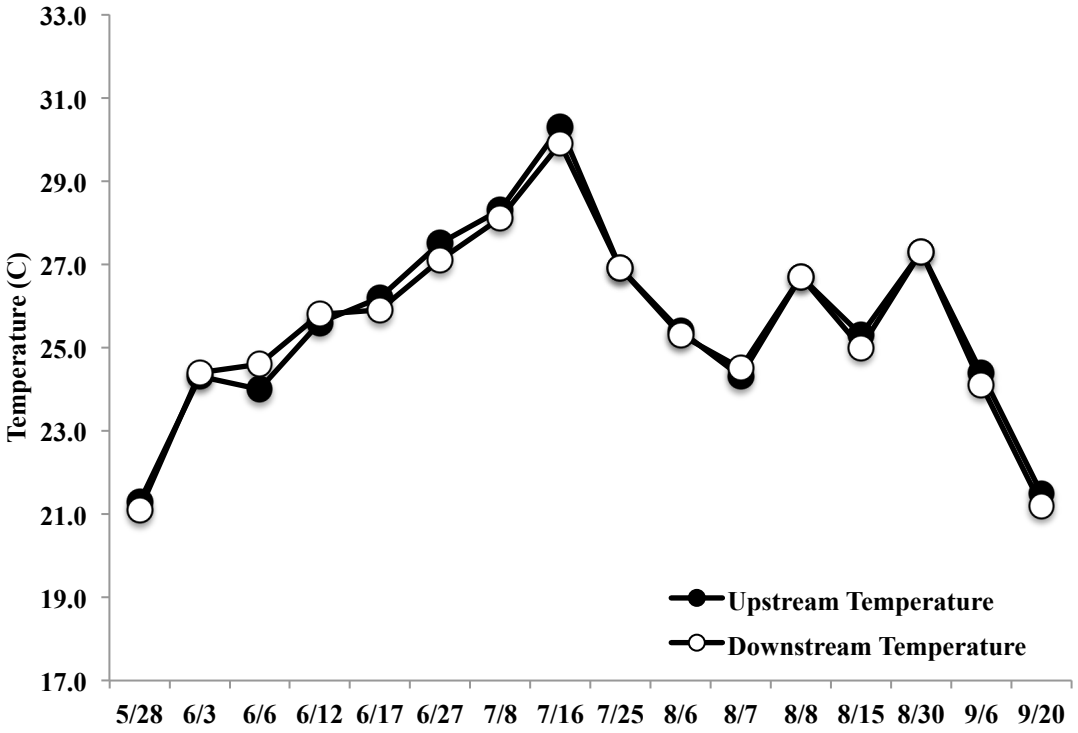


Figure 3.4: Upstream and downstream temperature (°C) at Marinetics oyster farm during Summer 2013.

Ammonia concentrations measured upstream and downstream of the aquaculture facility varied widely throughout the summer (Figure 3.5). Highest measures of ammonia were in the spring, while lowest measurements were taken later in the summer. Ammonia concentration was significantly higher downstream of the aquaculture facility ($p = 0.0014$). The average upstream concentration of ammonia was 1.053 $\mu molNH_3$ and the average downstream concentration was 1.879 $\mu molNH_3$.

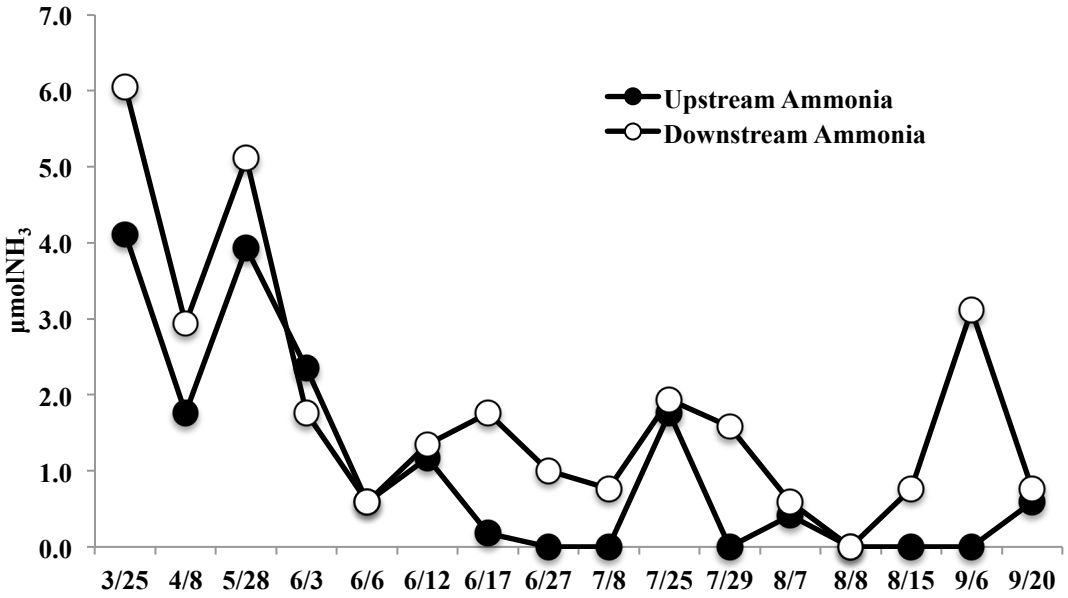


Figure 3.5: Upstream and downstream ammonia concentration (µmolNH_3) at Marinetics oyster farm during Spring/Summer 2013.

Linear regression analysis (Figure 3.6) showed no relationship between the change in concentration of ammonia and flow rate of water through the facility ($R^2 = 0.013$).

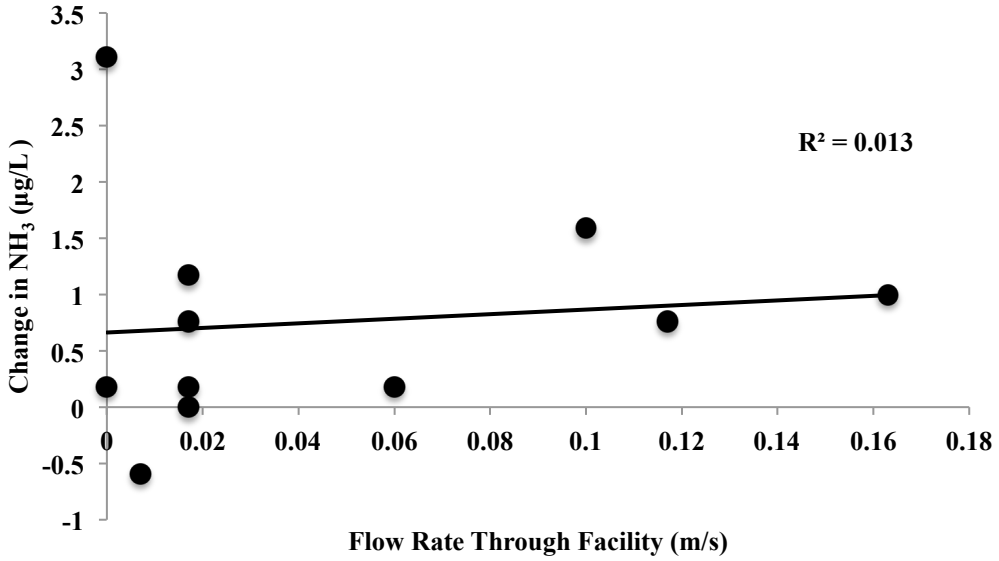


Figure 3.6: Comparison of change in NH_3 (µg/L) and flow rate (m/s) at the aquaculture facility during Summer 2013.

The concentration of nitrate did not change significantly from upstream to downstream ($p = 0.425$). The highest measured NO_3^- values were 3.23 µmol/L on April 8, and the lowest values were recorded in March (0.53 µmol/L upstream and no detectable NO_3^- downstream). Average upstream NO_3^- was 2.02 µmol/L and average downstream concentration was 2.08 µmol/L (Figure 3.7).

Nitrite concentration also showed no significant change as water passed through the facility ($p = 0.207$). The average upstream concentration of NO_2^- was 0.08 µmol/L and the average downstream concentration was 0.092 µmol/L (Figure 3.8). The highest measured value of NO_2^- was 0.217 µmol/L and the lowest measured value was 0.058 µmol/L.

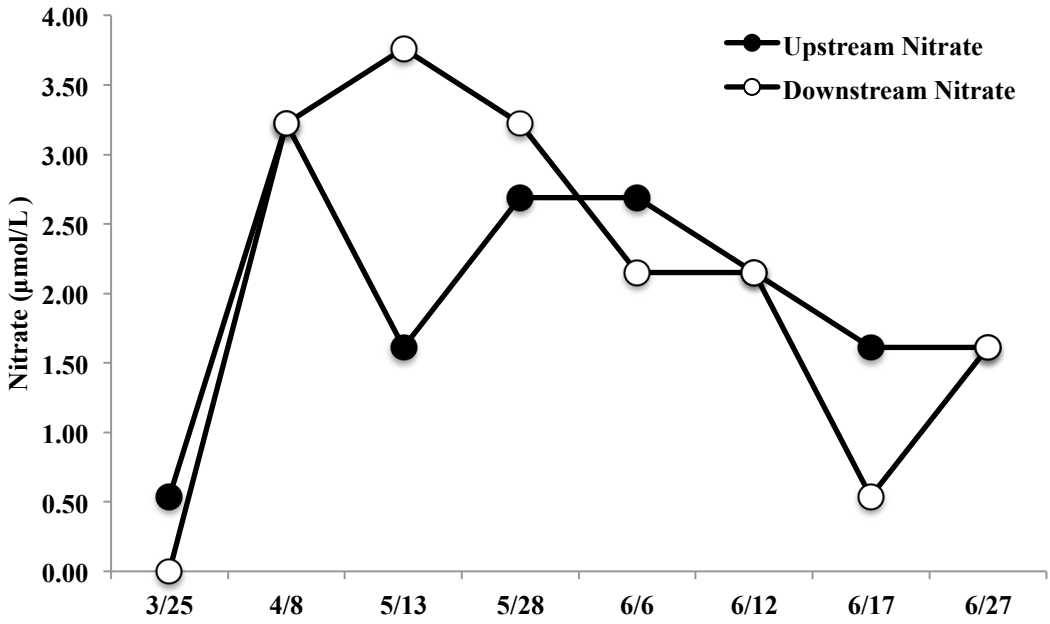


Figure 3.7: Upstream and downstream nitrate concentration (µmolNO_3/L) at Marinetics oyster farm during Spring/Summer 2013.

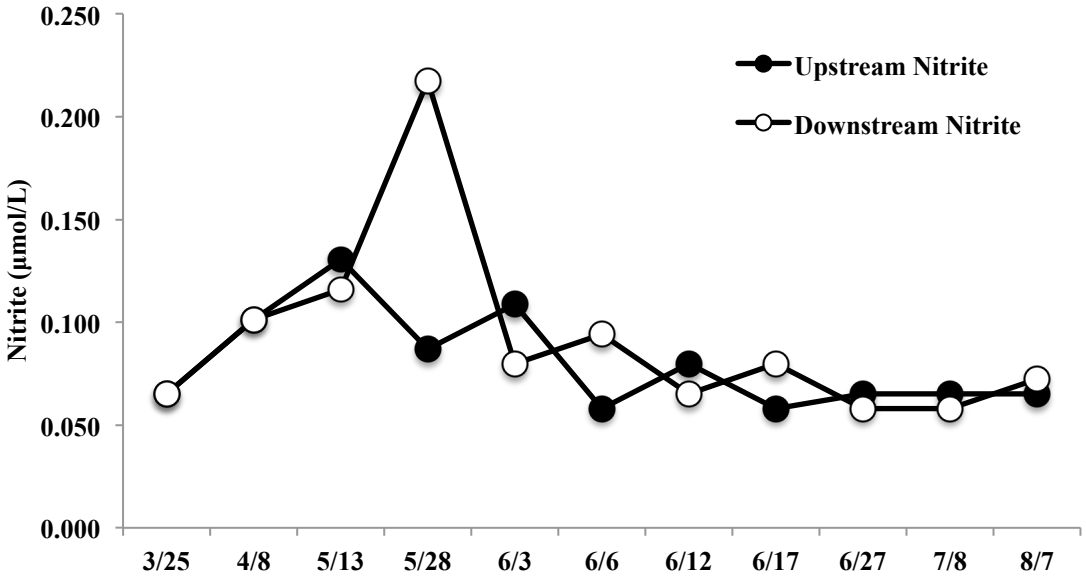


Figure 3.8: Upstream and downstream nitrite concentration (µmolNO_2/L) at Marinetics oyster farm during Spring/Summer 2013.

Phosphate concentration upstream and downstream of the facility varied throughout the sampling period (Figure 3.9), and did not change significantly from upstream to downstream ($p = 0.442$). Average upstream phosphate values were 0.696 µmolPO_4, while average downstream concentrations were 0.718 µmolPO_4. The results for phosphate were analyzed again excluding the two sampling dates containing changes in concentration much larger than all other dates (6/6 and 7/29), but the results were still statistically insignificant.

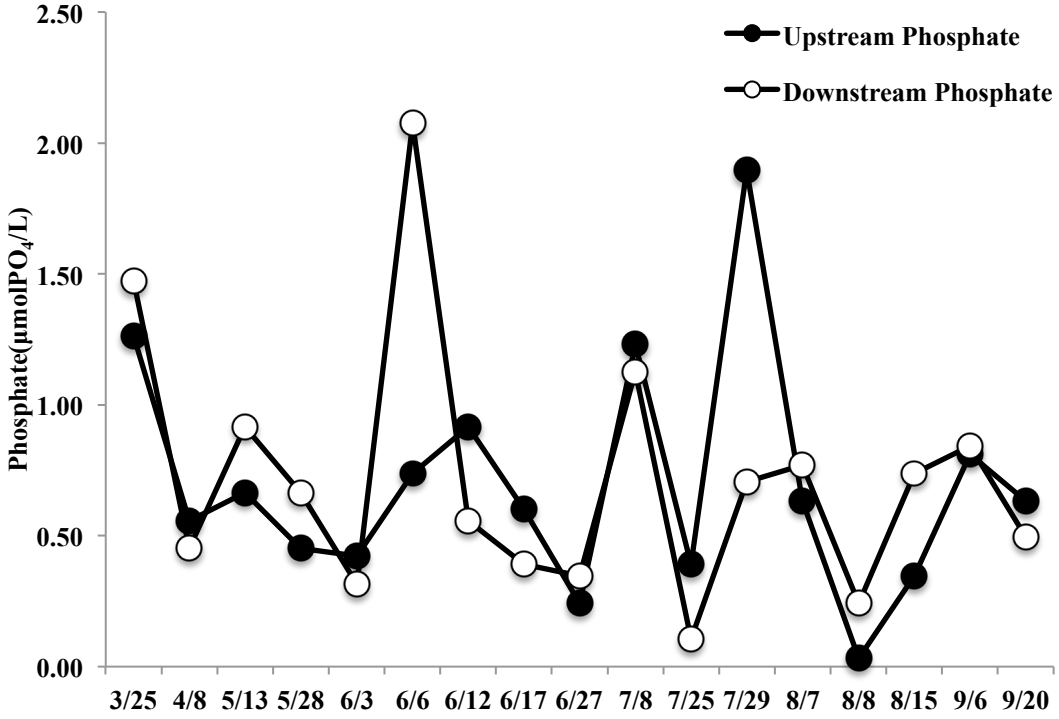


Figure 3.9: Upstream and downstream phosphate concentration (µmolPO_4) at Marinetics oyster farm during Spring/Summer 2013.

The concentration of chlorophyll-a (µg/L) was significantly lower ($p = 0.0449$) downstream of the aquaculture facility. Highest levels of chlorophyll-a were recorded in July, while the lowest levels of chlorophyll-a were recorded in May (Figure 3.10). The average upstream chlorophyll-a concentration was 118.94 µg/L and the average downstream concentration was 95.51 µg/L**.** Dates with higher levels of upstream chlorophyll-a often had the largest change in chlorophyll-a concentration as water passed through the aquaculture facility.

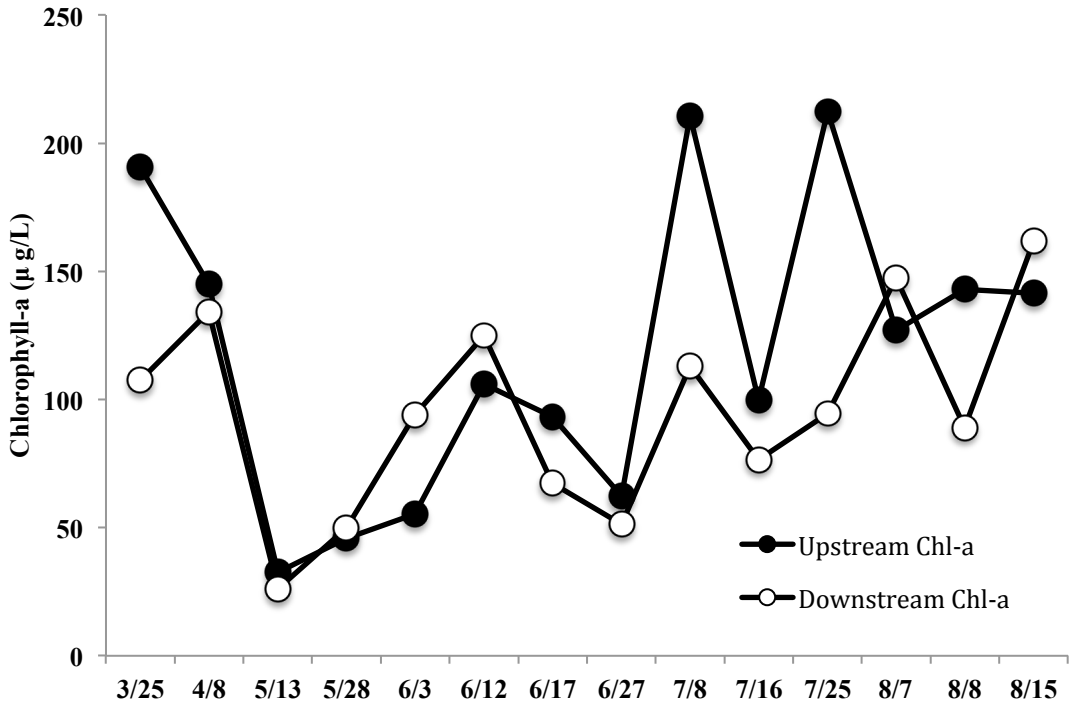


Figure 3.10: Upstream and downstream chlorophyll-a concentration (µg/L) at Marinetics oyster farm during Spring/Summer 2013.

The regression used to assess a relationship between change in Chl-a concentration and flow rate (Figure 3.11) showed no relationship ($R^2 = 0.006$). When change in Chl-a concentration from upstream to downstream was compared against the upstream concentration of Chl-a (Figure 3.12), a relationship was found demonstrating a greater change in concentration correlated with high levels of upstream Chl-a ($R^2 = 0.564$).

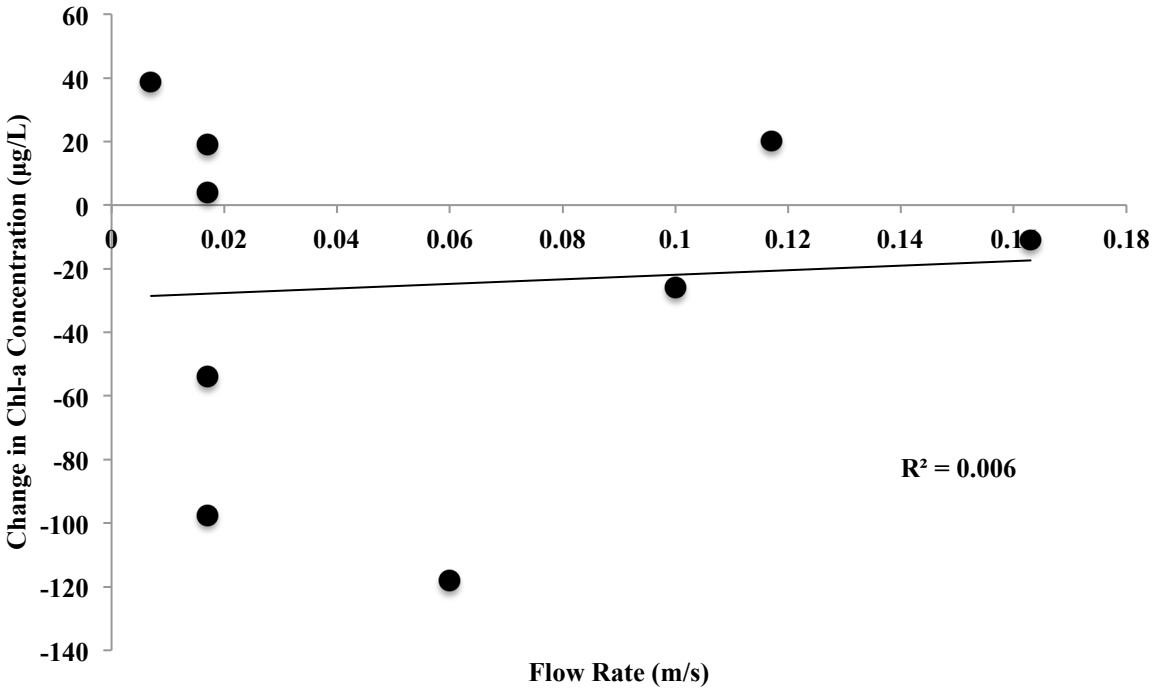


Fig 3.11: Relationship between flow rate and change in chlorophyll-a concentration from upstream to downstream.

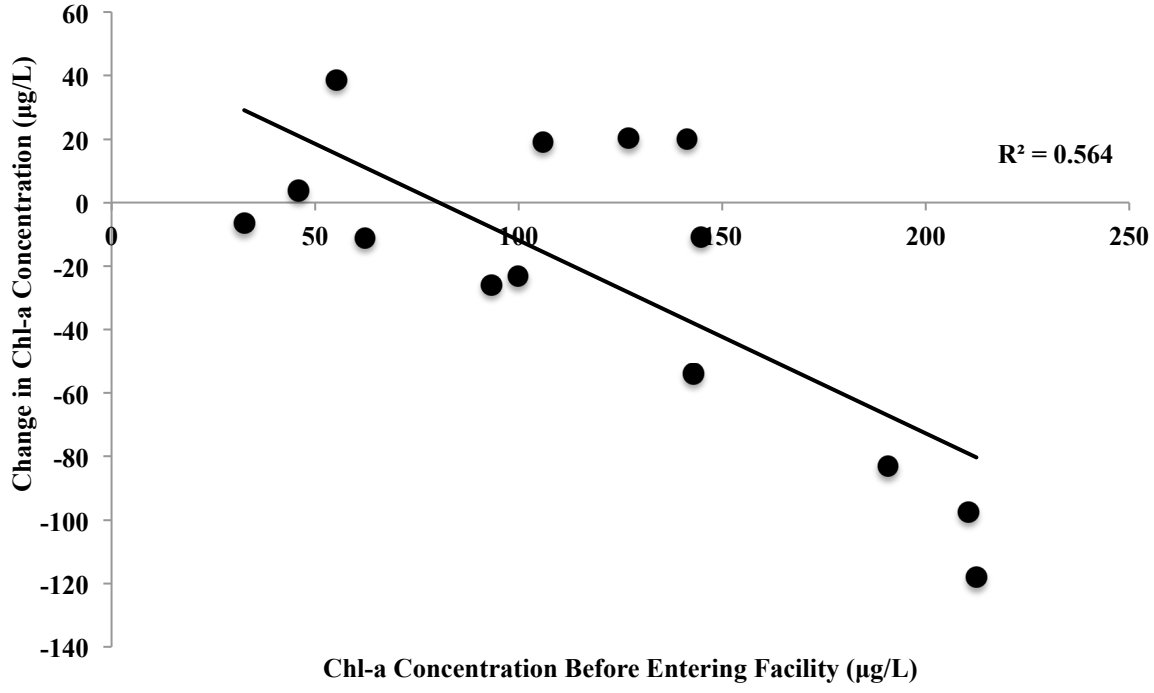


Fig 3.12: Relationship between chlorophyll-a concentration entering the aquaculture facility and total chlorophyll-a removal.

To determine if an increased level of upstream Chl-a would correspond with increased rates of ammonia excretion, the change in NH_3 concentration was regressed against the upstream Chl-a concentration (Figure 3.13). No correlation was found between these variables ($R^2 = 0.013$).

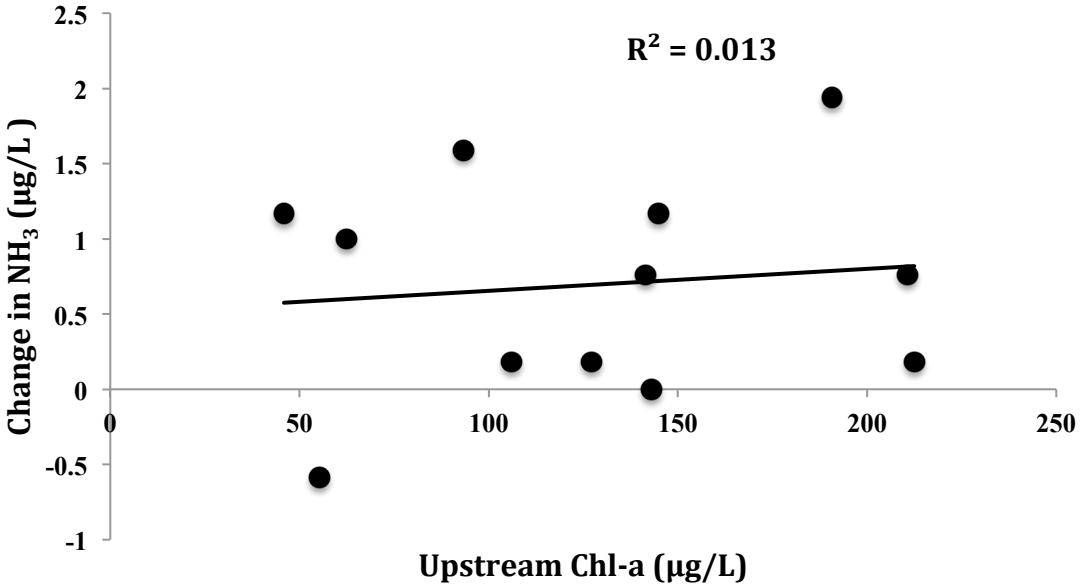


Fig 3.13: Relationship between chlorophyll-a concentration entering the aquaculture facility and change in NH_3 concentration from upstream to downstream.

3.1.3 Comparison of the Nutrient Prediction Model with Upstream/Downstream Data

The model developed to predict ammonia release by the aquaculture facility was reasonably accurate, and was within 5 µM on each date compared. Flow rate values used for model calculations can be seen in Table 3.2. Models for both hypothesized excretion rates (45.08 and 39.04) were not statistically different from the field-collected data (Figure 3.14). For the 45.08 model, $p = 0.685$, and for the 39.04 model $p = 0.413$.

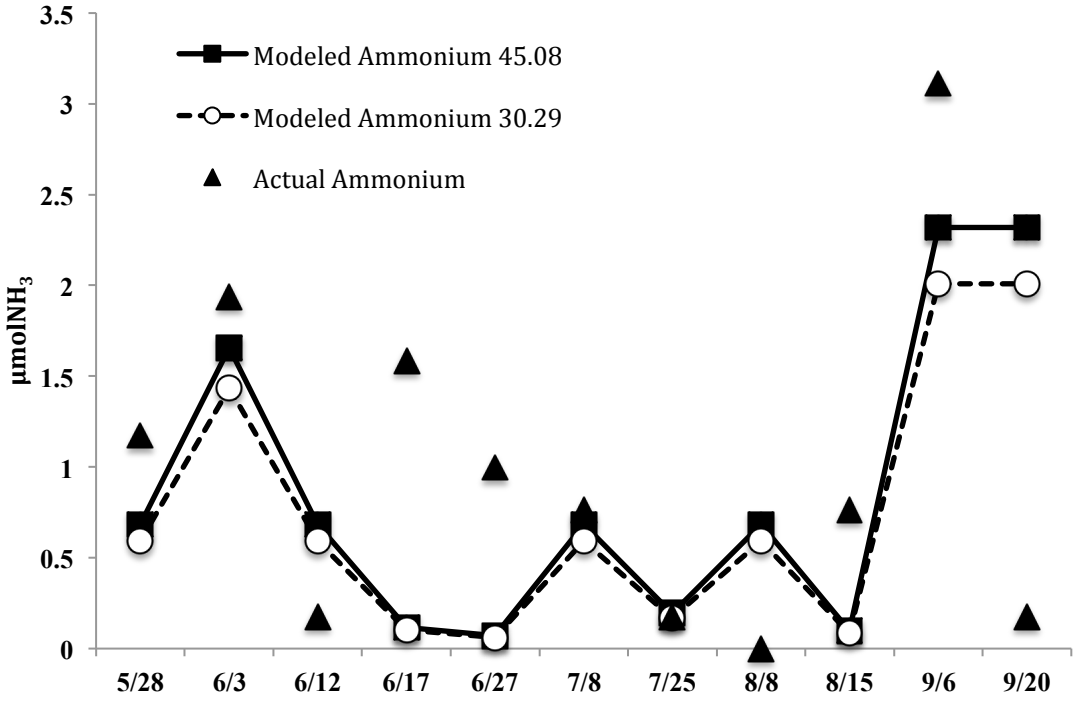


Figure 3.14: Modeled versus field collected change in ammonia concentration as water passes through Marinetics oyster farm, assuming ammonia excretion rates of 45.08 and 39.04 µmolNH_3/oyster*day^{-1}.

3.2 Performance of The Algal Turf Scrubber

3.2.1 Algal Turf Scrubber Growth Rates and Nutrient Content:

Growth rate was calculated as the harvested biomass divided by area per number of days since the last harvest, and is reported in terms of grams per square meter per day (g/m^2*d^{-1}). ATS harvest during summer 2012 averaged 110.54 g/m^2*d^{-1} (excluding greenwater), and productivity during summer 2013 averaged 82.8 g/m^2*d^{-1} (including greenwater harvest, but excluding data for ATS#2 between 8/8/13 and 8/30/13). Harvest data can be seen in Table 3.3.

The average nitrogen content for Summer 2012 harvests was 0.116 gN/100g of sample, which was used to calculate a removal rate of 9.61 gN/m^2*day^{-1}. The average phosphorus content for Summer 2012 harvests was 0.0024 gP/100g sample. The calculated daily phosphorus removal rate based on this average was 0.20 gP/m^2*day^{-1}. Algal Turf Scrubber harvest nitrogen and phosphorus content can be seen in Table 3.4.

The average percentage of the total harvest consisting of green water was 53%, with a range from 0-100% throughout Summer 2013 (Table 3.5). Harvest dates with 0% green water were those following pump failures, so there was no green water to be collected from the ATS, only the dried algae that remained attached to the screen. Green water was not collected during Summer 2012 harvests, so it can be reasonably assumed the total harvest would have been slightly higher if the green water was collected.

Table 3.3: Harvest data for ATS at the oyster aquaculture facility during Summer 2012 and 2013 growing seasons.

Harvest Date	ATS #	Harvested Algae (g)	Daily Harvest Rate (g/m^2*day^{-1})	Green Water (g)	Green Water Daily Harvest (g/m^2*day^{-1})	Total Biomass per Day (g/m^2*day^{-1})
9/17/12		310.14	53.65	-	-	-
9/24/12		831.06	143.77	-	-	-
10/1/12		550.25	95.19	-	-	-
10/15/12		1560.80	135.00	-	-	-
10/22/12		723.13	125.10	-	-	-
2012 Average		**795.08**	**110.5**	**-**	**-**	**-**
5/24/13		339.63	37.39	-	-	-
6/12/13		823.21	66.46	-	-	-
8/8/13	1	316.10	45.14	496.69	70.96	116.11
	2	-	-	-	-	-
	3	420.47	60.07	-	-	-
8/15/13	1	237.97	34.0	129.47	18.50	52.49
	2	-	-	-	-	-
	3	322.79	46.11	105.48	15.07	61.18
8/23/13	1	-	-	-	-	-
	2	-	-	-	-	-
	3	202.07	28.87	-	-	-
8/30/13	1	-	-	-	-	-
	2	-	-	-	-	-
	3	590.00	84.29	-	-	-
9/6/13	1	287.52	41.07	-	-	-
	2	-	-	-	-	-
	3	813.67	116.24	-	-	-
9/13/13	1	8.45	1.21	427.40	61.06	62.26
	2	41.66	5.95	622.06	88.87	94.81
	3	566.54	80.93	522.78	74.68	155.62
10/4/13	1	0	0	333.96	47.71	47.71
	2	0	0	848.77	121.25	121.25
	3	0	0	642.87	91.84	91.84
10/11/13	1	132.84	18.98	879.91	125.70	144.68
	2	-	-	-	-	-
	3	14.17	2.02	740.57	105.80	107.82
2013 Average		**284.28**	**37.11**	**522.73**	**74.68**	**82.8**
Two Season Average		**395.32**	**53.06**	**522.73**	**74.68**	**82.8**

Table 3.4: Nitrogen and phosphorus content of ATS harvest in late Summer 2012.

Date	Tissue N (gN/100g)	Tissue P (gP/100g)
9/17/12	0.141	0.004
9/24/12	0.083	0.001
10/1/12	0.132	0.003
10/15/12	0.078	0.002
10/22/12	0.145	0.002
Average	**0.116**	**0.0024**

Table 3.5: Percentage of ATS harvest consisting of green water.

Date	ATS#	Percentage of harvest as green water (%)
8/8/13	1	61
	3	0[1]
8/15/13	1	35
	3	25
8/23/13	3	0[1]
8/30/13	3	0[1]
9/6/13	1	0[1]
	3	0[1]
9/13/13	1	98
	2	94
	3	48
10/4/13	1	100
	2	100
	3	100
10/11/13	1	87
	3	98

[1] There was no green water for these samples as the pump had failed and the algae in the ATS had dried out. For these dates it was assumed that the pump failed earlier on the same day, as it is not possible to be sure of the actual time of failure.

3.2.2 Algal Turf Scrubber Total Volatile Solids

The five samples from Summer 2012 had an average volatile solids content of 3.8%. The ten samples from summer 2013 that were ashed had an average volatile solids content of 9.8%. Green water samples had a volatile solids content of 4.4%. Using these values the mass of algae in each harvest can be estimated (Table 3.6).

Table 3.6: Sample volatile solids content and estimated total algae mass in each harvest.

Harvest Date	ATS Replicate #	Harvest[1]	%Volatile Solids
9/17/12	-	310.14	3.3
9/24/12	-	831.06	4.2
10/1/12	-	550.25	3.7
10/15/12	-	1560.8	3.6
10/22/12	-	723.13	4.4
2012 Average			**3.8**
6/12/13	-	823.21	4.1
8/8/13	3	420.47	5.7
8/15/13	1	237.97	10.8
	3	322.79	10.4
8/23/13	3	202.07	15.7
8/30/13	3	590.00	11.3
9/6/13	3	813.67	11.2
9/13/13	2	41.66	6.8
	3	565.54	17.5
10/11/13	1	132.84	4.2
2013 Average			**9.8**
8/8/13	GW1	496.69	3.8
9/13/13	GW3	522.78	5.7
10/4/13	GW3	642.87	3.5
Greenwater Average			**4.4**

[1]Total harvest excluding green water (except the three tested green water samples). This value only takes into account the solid harvest remaining after sieving the harvested sample.

For Sumer 2013, the percent volatile solids value was compared against the number of weeks the ATS had been running without interruption by a broken pump. An increase in the percent volatile solids was found when the ATS had been continuously operational for a longer time period, with an R^2 value of 0.604 (Figure 3.15).

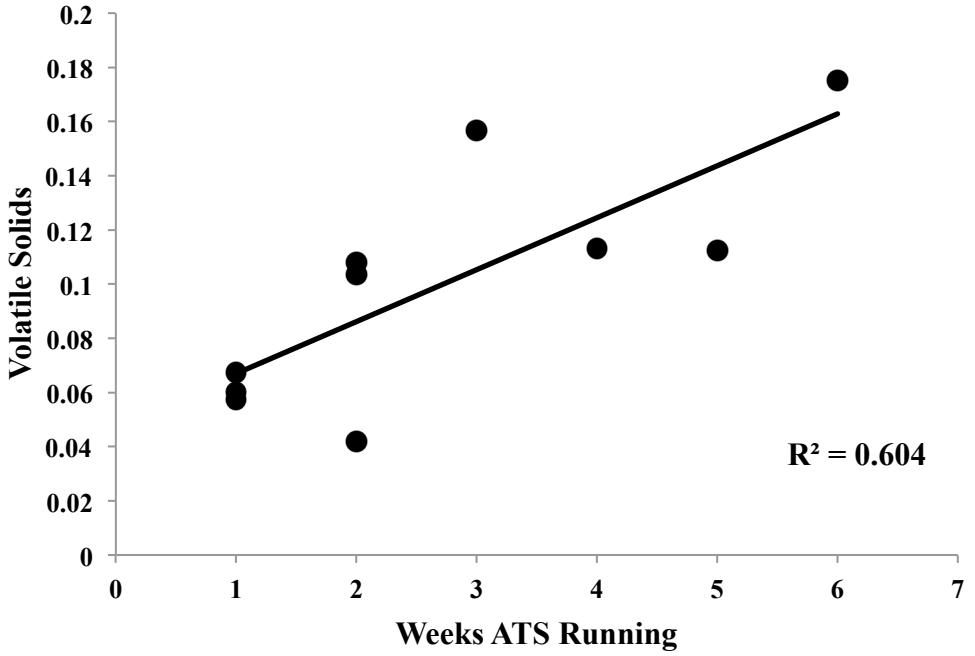


Figure 3.15: Correlation between consecutive weeks of ATS operation and volatile solids content of algal harvest.

3.2.3 Algal Species Composition:

The dominant algal species in the ATS throughout all growing seasons was *Ulva intestinalis*, a rapidly growing, green filamentous algae. Other dominant algae throughout the growing season include pennate diatoms and filamentous diatoms. Near the end of the first growing season, some patches of *Polysiphonia sp.* began to grow on the ATS screen.

CHAPTER IV: Discussion

4.1 Oyster Impact

4.1.1Oyster Excretion

As expected, the excretion rate of ammonia by oysters at the aquaculture facility was comparable to the rates found in previous studies using wild oysters. The average ammonia excretion rate determined in these experiments falls within the range of excretion rates determined in previous studies, and is slightly higher than the rates given by Srna and Baggaley (1976) and Hammen (1968), and lower than the rates given by Dame et al. (1992) and Pietros and Rice (2002)(Table 4.1).

Table 4.1: Comparison of ammonia excretion rates by *Crassostrea virginica* from previous studies with results from this study.

Study	**Calculated Excretion Rate ($\mu molNH_3/g\ dryweight*day^{-1}$)**
Srna and Baggaley (1976)	25
Hammen (1968)	11.7 – 38.328 ($\bar{x}$= 25.028)
Dame et al. (1992)	38.75
Pietros and Rice (2002)	67.39
This Study	**30.29**

These results demonstrate that triploid oysters in aquaculture do regenerate available nitrogen to the water column, but at rates comparable to wild, diploid oysters of the same species. It should be expected that excretion by oysters in an aquaculture facility affect water chemistry no differently than those found in a natural oyster reef, and that oysters in aquaculture have a similar influence on the excretion and deposition processes in the aquatic nitrogen cycle.

4.1.2 Upstream/Downstream Water Quality

Changes in water quality throughout Summer 2013 were as expected for each variable except for phosphate (Table 4.2). Dissolved oxygen and chlorophyll-a concentration decreased as water passed through the oyster aquaculture facility. Corresponding with this decrease was an increase in ammonia. Nitrate and nitrite concentrations remained the same, as did temperature and salinity. These results suggest an excellent location for an algal turf scrubber, as there is available nitrogen and a need to reintroduce oxygen back into the water column.

The relationship between an increase in Chl-a removal corresponding with a higher upstream concentration of Chl-a demonstrates the ability of oysters in aquaculture to effectively filter large quantities of water (Figure 3.12). The three sampling dates where the incoming Chl-a concentration was near 200 µg/L showed the greatest decrease in concentration downstream of the facility – almost half of the incoming concentration. Alongside this decrease, there was no relationship between rate of ammonia excretion and upstream Chl-a concentration (Figure 3.13), suggesting incorporation of phytoplankton N into the oyster shell and tissue, or increased production and deposition of feces and pseudofeces. Increased clearance of Chl-a at high concentrations does not coincide with increased rates of ammonia excretion. If water quality improvement is being considered when siting oyster aquaculture facilities, or reef restoration projects, then locations should be selected with areas of high Chl-a. Siting facilities at potential eutrophic locations with high Chl-a concentration would maximize removal of phytoplankton from the water column. The excretion rate of ammonia at sites with large

Table 4.2: Summary of predicted and recorded changes in water quality parameters as water passed through Marinetics oyster farm during Spring/Summer 2013.

Variable	Predicted Change	Actual Change	Upstream Average	Downstream Average	Upstream Range	Downstream Range	n
Dissolved Oxygen (mg/L)	Decrease	Decrease ($p = 0.0001$)	7.14	6.66	5.02 – 8.95	4.71 – 8.71	16
Salinity	None	None ($p = 0.30$)	10.27	10.28	8.7 – 12.3	8.8 – 12.3	16
Temperature (°C)	None	None ($p = 0.10$)	25.58	25.49	21.3 – 30.3	21.1 – 29.9	16
Ammonia (µmol/L)	Increase	Increase ($p = 0.0014$)	1.053	1.879	n/d[1] – 4.11	n/d – 8.22	16
Nitrate (µmol/L)	None	None ($p = 0.425$)	2.02	2.08	0.54 – 3.23	n/d – 3.76	8
Nitrite (µmol/L)	None	None ($p = 0.207$)	0.080	0.092	0.058 – 0.130	0.058 – 0.217	11
Phosphate (µmol/L)	Increase	None ($p = 0.442$)	0.696	0.718	0.242 – 1.895	0.105 – 2.074	17
Chlorophyll-a (µmol/L)	Decrease	Decrease ($p = 0.0449$)	118.94	95.51	32.58 – 212.45	26.15 – 161.64	14

[1]Indicates that the parameter being tested was below detectable limits.

phytoplankton stocks would likely not be high enough to simulate productivity back to the level found before water passed through the site.

Considering that nitrogen is the limiting nutrient in marine and brackish aquatic ecosystems (Smith, 1984), the increase of ammonia after water passed through the facility suggests opportunities for uptake and growth by phytoplankton, SAV, and macroalgae. The annual cycle of nutrient availability in the Chesapeake Bay supports the growth of diatoms in the spring when dissolved inorganic nitrogen is readily available near the surface of the water and there is high turbulence, shifting to growth of dinoflagellates and other motile species when surface nutrients are depleted, and are more available near the sediment (Baird and Ulanowicz, 1989; Patten et. al, 1963).

Oyster reefs may support a different phytoplankton community than water without oyster present by making more inorganic nitrogen readily available in the photic zone. The filtration process allows for light to penetrate deeper, and excretion by the oysters makes nitrogen readily available for uptake. Floating raft aquaculture should behave similarly to natural reefs when considering nutrient cycling, but most likely have a different influence on the local hydrography. Lenihan (1999) demonstrated that natural oyster reefs essentially "funnel" or compress the water flowing past into a narrower space, forcing higher flow rates as water passed over the reef. Oyster floats do not force water to flow through a smaller area, and results of this study showed no differences in flow rate from one part of the aquaculture facility to another, or differences between inside and outside the facility. Different flow regimes between oyster aquaculture and natural reefs could influence the ecologic community immediately downstream.

The "funneling" of water by natural oyster reefs concentrates food for the oysters, but forces the water to move over the reef more quickly. In this study, there was no relationship found between flow rate of water through the facility and Chl-a removal. There was however, an increase in removal of Chl-a corresponding to increased upstream loading, suggesting that even though water moves more rapidly through a natural oyster reef, at high Chl-a concentrations a similar reduction downstream should be expected when compared to reduction by floating raft aquaculture.

On 5 of the 14 dates Chl-a was measured at the aquaculture facility, an increase in concentration was recorded immediately downstream of the facility. This is somewhat surprising, as it would be expected that filtration by the oysters proceeds at a much more rapid rate than the growth rate of most phytoplankton species. Cyanobacteria populations may be able to double in size in a day, while diatoms and dinoflagellate populations take longer. There are a few possible suggestions for this change. The first is the flow rate and pattern of water through the facility. There was no correlation found between flow rate and Chl-a removal, the opposite of what was hypothesized. It makes sense that water moving more slowly through the facility would allow more time for oyster filtration. This idea was not supported, so the oysters either did not filter as efficiently at a lower flow rate, or there was some introduction of Chl-a into the downstream water.

Previous research has shown that bivalve excretion can lead to 100% increases in the doubling times of some phytoplankton species (Arzul et al., 2001). If water was flowing through the aquaculture facility at 0.01m/s (the lowest value recorded in this study; also the lowest value measurable by the flow meter used), it could take over an hour to move from upstream to downstream assuming an even flow through the whole

facility. On some dates in this study, flow rate was below measurable limits, suggesting an even longer potential residence time of water in the facility. On sampling dates with lower Chl-a loading rates, this could allow for doubling times of phytoplankton that could exceed the removal rate by oysters, depending on how water flows through the facility. Regressions showed that Chl-a removal and ammonia release in the facility are not correlated with flow rate, but Chl-a removal is dependent on upstream concentration of Chl-a. On a day with low flow (a high residence time of water) and low Chl-a concentration (and corresponding clearance rate), it is not unreasonable to assume that there may be a long enough residence time of water in the facility to see some increase in Chl-a concentration. The regression comparing upstream Chl-a concentration with change in ammonia also found no relationship, which could be due to production of phytoplankton within the facility. Further research on growth rates of different phytoplankton species after exposure to oyster excretion needs to be conducted to further elucidate this idea.

Other potential reasons for an increase in Chl-a concentration are intrusion of other water into the facility, the power washing of the oyster floats, or experimental error. Flow direction of water was measured at the same time as samples were taken, and the data collected do not suggest physical movement of Chl-a into the sampling area by the surrounding water body. The employees at the aquaculture facility do clean the oyster floats by power washing fairly frequently. Most of the floats develop large mats of cyanobacteria and other algae on them, and the runoff from the power washing does go back into the middle of the facility, which makes it a potential reason for the recorded increase.

4.1.3 Comparison of the Nutrient Prediction Model with Upstream/Downstream Data

The model developed predicted ammonia release by the aquaculture facility relatively accurately when considering the whole summer, but there were multiple dates where field recorded values were at least 2 – 3 times greater than projected. Possible causes for this include ammonia release by the sediment, a "funneling effect" by water flow patterns, or excess excretion by the oysters. Ammonia release by the sediment is most likely, as flow at the facility was found to be fairly uniform and thus unlikely to concentrate ammonia in specific areas. It was also found through the oyster excretion experiments that the oysters grown at the farm excrete ammonia at a rate comparable to previous studies, and this excretion rate was one that was used in the model. In this study, it was also shown that flow rate of water through the facility had no influence on the change in ammonia concentration (Figure 3.6).

Dame et al. (1992) used a field study to show that feces and pseudofeces deposition around mature reefs of *C. virginica* is responsible for approximately 60% of the ammonia flux in the surrounding water column (the other 40% is from oyster excretion). It can be expected that there are high rates of deposition of feces and pseudofeces at Marinetics oyster farm due to the high concentrations of chlorophyll-a measured throughout Summer 2013. One can also reasonably assume that oysters raised at the water's surface as opposed to the benthos will produce more feces and pseudofeces due to increased availability of phytoplankton in the photic zone. However, it would be reasonable to expect that the river current carries a large portion of feces and pseudofeces produced by the oysters at Marinetics downstream. This idea is supported early in the summer, when field results were higher than modeled results. On 6/17 and 6/27 there

were relatively high flow rates but very large changes in ammonia concentration as water passed through the facility.

A possible explanation for the decrease in change of ammonia concentration as the summer progressed is the growth of sessile algae on the oyster floats and other surfaces at the facility. To assess this possibility, algal growth on floats was estimated on three dates: 7/18/13, 7/25/13, and 8/8/13. Full details on these procedures can be found in the Appendix. The total dry estimated mass of algae ranged from 3 – 5.5 kg on each sampling date, enough biomass to remove significant amounts of ammonia that could be noticeable in the upstream/downstream study, and influence the accuracy of the model (Table 4.3). Employees at the oyster farm routinely removed oyster floats from the water to remove the attached algae by power washing, allowing for new growth, and continued removal of ammonia. Unfortunately, there were not enough sampling dates, or a detailed evaluation of the rate of removal of algae by power washing to determine nitrogen or phosphorus removal rates by this practice alone.

Table 4.3: Estimated algal biomass on oyster floats at Marinetics.

Date	Total Biomass (g)	Tissue N (gN/100g Algae)	Total N (g)
7/18/13	5467	2.22	121.37
7/25/13	3283	-	-
8/8/13	4634	1.92	88.97

The average total amount of nitrogen estimated to be held in the algae attached to the oyster floats at a given time was 105g (the percentage of nitrogen in the algae was determined to be 2.07gN/100 g algae). Since the rate of power-washing floats was not determined, only the average difference in attached algal biomass between weeks can be used to assume removal rates. The average change in biomass was 833g per week, or

119g/day for the whole facility. This amount of algal biomass would contain 22.49g of N. The amount of algae removed through power washing can be compared to the daily growth rate of the ATS in this study, 82.8g/m^2*day^{-1}. This demonstrates that the ATS is much more capable of removing large quantities of nitrogen in a given area through high rates of biomass production.

Chappelle et al. (2000) neglected to include benthic algae when attempting to model nitrogen and oxygen fluxes in a Mediterranean lagoon with intensive oyster aquaculture, and found that their model also overestimated ammonia concentrations for this reason.

4.2 Performance of the Algal Turf Scrubber

4.2.1 Nutrient Availability

Results of the water quality analysis at the oyster aquaculture facility show that nutrients in the form of ammonia and phosphate are readily available for algal uptake. This supports the notion of oyster reefs and aquaculture serving to recycle nutrients, while suggesting it is a good choice of location to implement an ATS, or other method of IMTA. Previous work regarding ATS productivity and nutrient removal capabilities shows that both can be increased when the ATS are installed at a point source with high nutrient loading (Table 1.2). In Mulbry et al. (2008), this was demonstrated by comparing ATS productivity at low and high rates of loading with dairy manure. Under higher manure loading rates, ATS productivity increased by an order of magnitude from 2.5 g/m^2*day^{-1}, to 25.0 g/m^2*day^{-1}. The tissue nitrogen content of the harvested algae

was 7.0 gN/m^2*day^{-1} at the high loading rate, and 4.3 gN/m^2*day^{-1} at the lower loading rate. Tissue phosphorus content was also higher in algae harvested from ATS with the high manure loading rate. Kebede-Westhead et al. (2003), found similar results with high and low loading rates of anaerobically digested dairy manure.

While it may be impractical to compare the nutrient release by an aquaculture facility to that of manure from a dairy farm, it nevertheless shows that it can be expected that ATS installed near point sources such as oyster aquaculture facilities will have improved productivity and bioremediation ability. Rural areas do not offer as many point sources of nutrients as urban areas, but it is more practical to install ATS in these areas, as they require large areas of land, which can be expensive in urban areas. Integration of ATS with industry such as aquaculture, which is often conducted in more rural areas could make implementation of the technology more practical through siting at a point source, as well as in a location with cheaper land.

4.2.2 Algal Species Composition and Greenwater Content

An important aspect of the ATS is the ability to remove harvested algae and nutrients from the surrounding aquatic ecosystem. This is accomplished by creating an environment that supports the growth of sessile algal species, which are able to attach to the screen in the ATS raceway. Throughout most of the growing season, filamentous diatoms and *Ulva intestinalis* – both sessile species, dominated the ATS. These algae were also found growing on the floats around the aquaculture facility. High wave energy and frequent harvest of the ATS precluded colonization and growth by some of the other

algal species that were common throughout the facility, such as *Ulva lactuca* and *Calothrix* spp, but colonies of *Polysiphonia* were witnessed towards the end of both growing seasons.

While current and previous studies using the ATS have allowed for colonization of the system naturally, the potential for seeding the screen in the raceway with a more economically valuable algal species, or a species with high growth and nutrient removal rates are potential methods to improve the efficiency and broader implementation of the technology. Methods for rope culture of algal species, including methods to seed ropes with a single species, have been practiced in Japan and China for centuries for culture of multiple algal species including *Porphyra*, which is eaten as nori (Oohusa, 1984 and 1993). Research to develop methods of algal aquaculture in Long Island Sound began in the 1980's, with a main focus on *Laminaria longricruris* and *Porphyra* (Yarish and Egan, 1987 and 1989; Yarish et al., 1998). More recently, Li et al. (2014) investigated methods for cultivating *Ulva intestinalis* seed stock in the Chesapeake Bay, along with studying the growth rate of *Gracilaria* in culture alongside the same facility investigated in this study (Li et al., 2013).

The percentage of the ATS harvest as green water was highest on harvest dates one week after there were pump issues. This is likely due to the community structure of algae in the ATS consisting of more diatoms than chlorophytes, which take slightly longer to become established within the system. Usually, diatoms are more common in cooler seasons in an established ATS (Adey et al., 2013), so it can be hypothesized that if there were fewer disruptions by pump failure, there would be more green macroalgae growing in the system during the summer. A different algal community could lead to

changes in total harvest, nutrient content, ash content, and the amount of total harvest as green water.

4.2.3 Algal Turf Scrubber Growth Rates and Nutrient Uptake

Results of this study compared to other ATS studies conducted in the Chesapeake Bay region (Table 1.2) demonstrate high productivity rates (82.8 g/m^2*day^{-1}), along with correspondingly high rates of nutrient removal. Productivity of the ATS located at the oyster farm exceeded or was comparable to studies conducted using water with very high nutrient content in the form of dairy and swine manure (Kebede-Westhead et al., 2003, 2006; Mulbry et al., 2008; Mulbry and Wilkie, 2001; Wilkie and Mulbry, 2002). These results suggest that high levels of nutrients may not be the contributing factor to increased growth rates in the ATS at the oyster aquaculture facility, and there may be another reason. Previous studies have suggested that oyster reef restoration may cause localized macroalgae blooms (Newell, 2005), and others have shown that there may be an organic compound either excreted by oysters, or found in their pseudofeces that promotes the growth of certain algal species (Arzul et al., 2001; Cognie and Barille, 1999). Other possibilities that could contribute to higher algal productivity rates include decreased competition for nutrients by phytoplankton through oyster filtration, or increased availability of carbon through oyster respiration.

The primary purpose of the ATS is nutrient removal, and the main objective of this study was to assess the nutrient removal potential of ATS at an oyster aquaculture facility. Nutrient removal rates were calculated by multiplying the percentage of tissue N

or P by the productivity rate, to give a removal rate of the given nutrient per area per day (gN/m^2*d^{-1} or gP/m^2*d^{-1}). By using this data, it was possible to calculate the necessary size needed for an ATS to remediate all of the nitrogen excreted by the oysters at the facility. A daily oyster nitrogen excretion value of $6.6x10^{-4}$ gN/oyster*d^{-1} was estimated from the average rate found in of four previous studies investigating oyster excretion (Srna and Baggaley, 1976; Hammen, 1968; Pietros and Rice, 2002; Dame et al., 1992). For this estimation, it was assumed that all oysters at the facility had an equivalent dry tissue biomass of 1.0g, which is about market size for an oyster. By multiplying the oyster excretion rate value by 10^6 (the estimated number of live oysters at the aquaculture facility), a daily nitrogen excretion rate of approximately 6600 gN/day can be assumed. Dividing this value by the average N assimilation rate of the ATS, it can be estimated that an ATS of 687.5 m^2 would be necessary to remove all of the nitrogen that was remineralized through oyster filtration and excretion. Multiplying this area by the average productivity allows for the estimation that an ATS of this size would produce approximately 56.93kg of algal biomass per day (dry weight). This ATS would also remove 137.4 g of phosphorus from the surrounding ecosystem daily.

Similarly, it is possible to calculate the size of an ATS needed to remediate all of the phosphorus excreted by oysters at the aquaculture facility. Using a phosphorus excretion rate of .245 μg/oyster*hr^{-1} (Satomi and Pomeroy, 1965) it was estimated that the aquaculture facility released approximately 58.8 g of available phosphorus per day. An ATS of 294 m^2 would be of sufficient size to remediate this phosphorus, and would also remove 2.8 kg of nitrogen per day – less than half the N of the ATS scaled to remediate all the nitrogen excreted by the oysters at the facility.

4.2.4 Algal Turf Scrubber Percent Organic Content

Results regarding the volatile solids of the ATS harvest show that the total mass of solids harvested could be misleading as far as inferring the total algal growth. The large amount of sediment trapped by these systems is responsible for the low volatile solids content recorded in this study. It is difficult to manage or reduce the amount of sediment entering into the ATS in a location such as Marinetics where the water is turbulent and tidal ranges require the intake pipe for the system to be close to the benthos in order to remain submerged during low tide. The sediments harvested with the algae do contain nutrients, and could also potentially be important as remediation towards the state of Maryland Total Maximum Daily Load for sediment. Strictly in terms of nutrient removal, maximizing the total harvest will increase the daily removal rate for N and P.

If the main purpose of the ATS is to produce biomass for removal of N, it is critical to maintain water flow in order to maximize the algal content of the harvest (Figure 3.15). A longer ATS raceway would likely allow for more sediment deposition near the dump bucket, and an increased proportion of algal growth over sediment towards the bottom of the raceway. The small size of the ATS used in this study may magnify the results assessing change in volatile solids content compared to time the ATS was operated, as well as in assumptions regarding nitrogen uptake by sediment deposited in the system.

As mentioned previously, a different algal community could yield different nutrient removal rates, along with different ash content. It can be expected that an ATS harvest consisting green macroalgae would likely have a higher percentage of total organic material than a same sized harvest made up of diatoms.

4.2.5 Difficulties with the ATS Pump

Throughout both growing seasons, sediment and other debris frequently clogged the pool pump that was used to supply water to all three ATS. To counter this problem, a small submersible pump was installed for each individual ATS. The submersible pumps were put inside a mesh bag to keep out large debris, and were able to provide enough of a flow of water to keep the algae growing in the ATS alive, but not enough to cause the dump bucket to tip more than once a minute (the pool pump caused the dump buckets to tip every 8-10 seconds). Unfortunately, clogging was also a problem with these pumps, as nearly every week they were filled with sediment. Being able to maintain flow of water through the ATS would have given more consistent results, but the location of the study site and the lack of funding available made it impossible to visit the site more than once per week.

Chapter V: Conclusions and Suggestions for Further Research

5.1.1 Conclusions Regarding Immediate Impact of Oyster Aquaculture on Surrounding Water Column

Results of this study demonstrate the impact of commercial scale oyster-aquaculture on the biochemistry of the water column. In this case, a significant increase in available ammonia, coupled with significant decreases in chlorophyll-a and dissolved oxygen was observed downstream of the aquaculture facility. As the oyster aquaculture industry continues to expand in the Chesapeake Bay, the short-term impact on the water body in close proximity to individual aquaculture operations needs to be considered when siting and regulating this type of facility.

Investigating the change in phytoplankton community structure and rejuvenation downstream of oyster aquaculture facilities is a logical next step in understanding how oyster aquaculture compares to natural reefs in terms of the biological impact. Water flow dynamics and geochemistry of these aquaculture facilities also needs to be explored in regards to feces and pseudofeces deposition and the subsequent sedimentary processes.

5.1.2 Conclusions Regarding Implementation of the Algal Turf Scrubber at an Aquaculture Facility

This may be the first demonstration of integrated multi-trophic aquaculture in the Chesapeake Bay, and the productivity and nutrient removal by the Algal Turf Scrubbers at the oyster aquaculture facility showed that integrated aquaculture is possible in the region, and can be used as a method for nutrient bioremediation. Integrating ATS with

aquaculture facilities is a method that can be used to maximize nutrient bioremediation by the technology.

Further studies investigating the potential for IMTA in the Chesapeake Bay have already been started, and will be important as the aquaculture industry continues to grow in the region. The ability to control growth of individual species of economic value, such as *Porphyra* or *Gracilaria*, will be critical for this development. By finding more uses for the harvested algae, the practicality of implementing ATS technology around the Chesapeake Bay will increase.

Appendices:

Appendix A: Methods for Assessing Algal Growth on Oyster Floats

In an attempt to estimate the amount of attached algal biomass on the oyster floats at Marinetics, a simple qualitative method was developed. Floats were assigned a rating of either "clean", "light", "medium", or "heavy". Clean floats were those that had recently been powerwashed, and had no visible algae attached. Light floats were those with minimal algal growth, with no more than 20% of the float having algae of any length attached. Medium was defined as floats with between 20-60% coverage by algae, and those with large sheets of *U. lactuca* growing inside. Heavy was defined as any float with greater than 60% coverage. All floats were visually assessed and recorded on three dates. On each date, three floats from the light, medium, and heavy classification were randomly chosen and had all algae removed by scraping. This algae was returned to the lab, and dried in the same manner as the ATS harvested algae. The mass of algae for each qualitative group was assumed to be an accurate representation of all other floats assigned to that group. By multiplying the representative mass by the total number of floats in a given qualitative group, an estimate of the total float biomass could be made. Tissue N content was assessed for two of the sample dates following the same methods described for the algal turf scrubber samples.

Appendix B:

Dominant algal species found on oyster floats at Marinetics oyster aquaculture facility.

Species	Phylum
Ulva intestinalis	Chlorophyta
Ulva lactuca	Chlorophyta
Ectocarpus spp.	Heterokontophyta
Polysiphonia spp.	Rhodophyta
Cladophora spp.	Chlorophyta
Melosira nummuloides	Heterokontophyta
Rhizoclonium spp.	Chlorophyta
Ceramium spp.	Rhodophyta
Gracilaria spp.	Rhodophyta
Calothrix spp.	Cyanobacteria (Blue-green algae)

Appendix C:

Sample	TKN (gN/100g)	P (gP/100g)
Control 1a	3.350	0.0067
Control 1b	2.650	0.0050
Control 1c	2.750	0.0067
Control 2a	2.578	0.0156
Control 2b	2.391	0.0109
Control 2c	2.438	0.0141
Control 3a	2.500	0.0097
Control 3b	3.167	0.0111
Control 3c	2.667	0.0125
Average	**2.721**	**0.010**
Test 1a	3.000	0.0191
Test 1b	2.912	0.0191
Test 1c	2.956	0.0206
Test 2a	3.281	0.0141
Test 2b	3.047	0.0172
Test 2c	3.188	0.0172
Test 3a	2.880	0.0092
Test 3b	3.160	0.0092
Test 3c	2.880	0.0092
Average	**3.034**	**0.0150**

Results of a preliminary study investigating tissue N and P content of *Ulva intestinalis* grown in cages inside ("Test") and outside the aquaculture facility ("Control"). Results for both N and P content showed that tissue nutrient content was higher in algae grown inside the oyster farm ($P<0.05$).

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